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(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number:

0 388 232

A1

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(12)

EUROPEAN PATENT APPLICATION

(21) Application number: 90302866.0

(51) Int. Cl.5: C12N 15/51, A61K 39/29,
G01N 33/576, C12Q 1/70

(22) Date of filing: 16.03.90

(23) Priority: 17.03.89 US 325338
20.04.89 US 341334
18.05.89 US 355002

(24) Date of publication of application:
19.09.90 Bulletin 90/38

(25) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

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(54) NANBV diagnostics and vaccines.

(57) A new virus, Hepatitis C virus (HCV), which has proven to be the major etiologic agent of blood-borne NANBH, was discovered by Applicant. The initial work on this virus, which includes a partial genomic sequence of the prototype HCV isolate, is described in EPO Pub. No. 318.216, and PCT Pub. No. WO/89/04669. The present invention, which in part is based on new HCV sequences and polypeptides which are not disclosed in the above-cited publications, includes the application of these new sequences and polypeptides in immunoassays, probe diagnostics, anti-HCV antibody production, PCR technology, and recombinant DNA technology. Included within the invention also are novel immunogenic polypeptides encoded within clones containing HCV cDNA, novel methods for purifying an immunogenic HCV polypeptide, and antisense polynucleotides derived from HCV cDNA.

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NANBV DIAGNOSTICS AND VACCINES

Technical Field

The invention relates to materials and methodologies for managing the spread of non-A, non-B hepatitis virus (NANBV) infection. More specifically, it relates to polynucleotides derived from the genome of an etiologic agent of NANBH, hepatitis C virus (HCV), to polypeptides encoded therein, and to antibodies directed to the polypeptides. These reagents are useful as screening agents for HCV and its infection, and as protective agents against the disease.

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- PCT Pub. No. WO 89/04669
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- 55 U.S. Patent No. 4,444,887
- U.S. Patent No. 4,466,917
- U.S. Patent No. 4,472,500
- U.S. Patent No. 4,491,632

U.S. Patent No. 4,493,890

Background Art

5 Non-A, Non-B hepatitis (NANBH) is a transmissible disease or family of diseases that are believed to be viral-induced, and that are distinguishable from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), and delta hepatitis virus (HDV), as well as the hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus 10 (EBV). NANBH was first identified in transfused individuals. Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH is due to a transmissible infectious agent or agents.

15 Epidemiologic evidence is suggestive that there may be three types of NANBH: the water-borne epidemic type; the blood or needle associated type; and the sporadically occurring (community acquired) type. However, the number of agents which may be the causative of NANBH are unknown.

20 Clinical diagnosis and identification of NANBH has been accomplished primarily by exclusion of other viral markers. Among the methods used to detect putative NANBV antigens and antibodies are agar-gel diffusion, counterimmunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay. However, none of these assays has proved to be sufficiently sensitive, specific, and reproducible to be used as a diagnostic test for NANBH.

25 Previously there was neither clarity nor agreement as to the identity or specificity of the antigen antibody systems associated with agents of NANBH. This was due, at least in part, to the prior or co-infection of HBV with NANBV in individuals, and to the known complexity of the soluble and particulate antigens associated with HBV, as well as to the integration of HBV DNA into the genome of liver cells. In addition, there is the possibility that NANBH is caused by more than one infectious agent, as well as the 30 possibility that NANBH has been misdiagnosed. Moreover, it is unclear what the serological assays detect in the serum of patients with NANBH. It has been postulated that the agar-gel diffusion and counterimmunoelectrophoresis assays detect autoimmune responses or nonspecific protein interactions that sometimes occur between serum specimens, and that they do not represent specific NANBV antigen-antibody reactions. The immunofluorescence, and enzyme-linked immunosorbent, and radioimmunoassays appear to detect low levels of a rheumatoid-factor-like material that is frequently present in the serum of patients with NANBH as well as in patients with other hepatic and nonhepatic diseases. Some of the reactivity detected may represent antibody to host-determined cytoplasmic antigens.

35 There have been a number of candidate NANBV. See, for example the reviews by Prince (1983), Feinstone and Hoofnagle (1984), and Overby (1985, 1986, 1987) and the article by Iwarson (1987). However, there is no proof that any of these candidates represent the etiological agent of NANBH.

40 The demand for sensitive, specific methods for screening and identifying carriers of NANBV and NANBV contaminated blood or blood products is significant. Post-transfusion hepatitis (PTH) occurs in approximately 10% of transfused patients, and NANBH accounts for up to 90% of these cases. The major problem in this disease is the frequent progression to chronic liver damage (25-55%).

45 Patient care as well as the prevention of transmission of NANBH by blood and blood products or by close personal contact require reliable screening, diagnostic and prognostic tools to detect nucleic acids, antigens and antibodies related to NANBV. In addition, there is also a need for effective vaccines and immunotherapeutic therapeutic agents for the prevention and/or treatment of the disease.

50 Applicant discovered a new virus, the Hepatitis C virus (HCV), which has proven to be the major etiologic agent of blood-borne NANBH (BB-NANBH). Applicant's initial work, including a partial genomic sequence of the prototype HCV isolate, CDC/HCV1 (also called HCV1), is described in EPO Pub. No. 318,216 (published 31 May 1989) and PCT Pub. No. WO 89/04669 (published 1 June 1989). The disclosures of these patent applications, as well as any corresponding national patent applications, are incorporated herein by reference. These applications teach, inter alia, recombinant DNA methods of cloning and expressing HCV sequences, HCV polypeptides, HCV immunodiagnostic techniques, HCV probe diagnostic techniques, anti-HCV antibodies, and methods of isolating new hCV sequences, including sequences of new HCV isolates.

55

Disclosure of the Invention

The present invention is based, in part, on new HCV sequences and polypeptides that are not disclosed

in EPO Pub. No. 318,216, or in PCT Pub. No. WO 89/04669. Included within the invention is the application of these new sequences and polypeptides in, inter alia, immunodiagnostics, probe diagnostics, anti-HCV antibody production, PCR technology and recombinant DNA technology. Included within the invention, also, are new immunoassays based upon the immunogenicity of HCV polypeptides disclosed herein. The new subject matter claimed herein, while developed using techniques described in, for example, EPO Pub. No. 318,216, has a priority date which antecedes that publication, or any counterpart thereof. Thus, the invention provides novel compositions and methods useful for screening samples for HCV antigens and antibodies, and useful for treatment of HCV infections.

Accordingly, one aspect of the invention is a recombinant polynucleotide comprising a sequence derived from HCV cDNA, wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

Another aspect of the invention is a purified polypeptide comprising an epitope encoded within HCV cDNA wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

Yet another aspect of the invention is an immunogenic polypeptide produced by a cell transformed with a recombinant expression vector comprising an ORF of DNA derived from HCV cDNA, wherein the HCV cDNA is comprised of a sequence derived from the HCV cDNA sequence in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the ORF is operably linked to a control sequence compatible with a desired host.

Another aspect of the invention is a peptide comprising an HCV epitope, wherein the peptide is of the formula

25 AA_x-AA_y,

wherein x and y designate amino acid numbers shown in Fig. 17, and wherein the peptide is selected from the group consisting of AA1-AA25, AA1-AA50, AA1-AA84, AA9-AA177, AA1-AA10, AA5-AA20, AA20-AA25, AA35-AA45, AA45-AA100, AA40-AA90, AA45-AA65, AA65-AA75, AA80-90, AA99-AA120, AA95-AA110, AA105-AA120, AA100-AA150, AA150-AA200, AA155-AA170, AA190-AA210, AA200-AA250, AA220-AA240, AA245-AA265, AA250-AA300, AA290-AA330, AA290-305, AA300-AA350, AA310-AA330, AA350-AA400, AA380-AA395, AA405-AA495, AA400-AA450, AA405-AA415, AA415-AA425, AA425-AA435, AA437-AA582, AA450-AA500, AA440-AA460, AA460-AA470, AA475-AA495, AA500-AA550, AA511-AA690, AA515-AA550, AA550-AA600, AA550-AA625, AA575-AA605, AA585-AA600, AA600-AA650, AA600-AA625, AA635-AA665, AA650-AA700, AA645-AA680, AA700-AA750, AA700-AA725, AA700-AA750, AA725-AA775, AA770-AA790, AA750-AA800, AA800-AA815, AA825-AA850, AA850-AA875, AA800-AA850, AA920-AA990, AA850-AA900, AA920-AA945, AA940-AA965, AA970-AA990, AA950-AA1000, AA1000-AA1060, AA1000-AA1025, AA1000-AA1050, AA1025-AA1040, AA1040-AA1055, AA1075-AA1175, AA1050-AA1200, AA1070-AA1100, AA1100-AA1130, AA1140-AA1165, AA1192-AA1457, AA1195-AA1250, AA1200-AA1225, AA1225-AA1250, AA1250-AA1300, AA1260-AA1310, AA1260-AA1280, AA1266-AA1428, AA1300-AA1350, AA1290-AA1310, AA1310-AA1340, AA1345-AA1405, AA1345-AA1365, AA1350-AA1400, AA1365-AA1380, AA1380-AA1405, AA1400-AA1450, AA1450-AA1500, AA1460-AA1475, AA1475-AA1515, AA1475-AA1500, AA1500-AA1550, AA1500-AA1515, AA1515-AA1550, AA1550-AA1600, AA1545-AA1560, AA1569-AA1931, AA1570-AA1590, AA1595-AA1610, AA1590-AA1650, AA1610-AA1645, AA1650-AA1690, AA1685-AA1770, AA1689-AA1805, AA1690-AA1720, AA1694-AA1735, AA1720-AA1745, AA1745-AA1770, AA1750-AA1800, AA1775-AA1810, AA1795-AA1850, AA1850-AA1900, AA1900-AA1950, AA1900-AA1920, AA1916-AA2021, AA1920-AA1940, AA1949-AA2124, AA1950-AA2000, AA1950-AA1985, AA1980-AA2000, AA2000-AA2050, AA2005-AA2025, AA2020-AA2045, AA2045-AA2100, AA2045-AA2070, AA2054-AA2223, AA2070-AA2100, AA2100-AA2150, AA2150-AA2200, AA2200-AA2250, AA2200-AA2325, AA2250-AA2330, AA2255-AA2270, AA2265-AA2280, AA2280-AA2290, AA2287-AA2385, AA2300-AA2350, AA2290-AA2310, AA2310-AA2330, AA2330-AA2350, AA2350-AA2400, AA2348-AA2464, AA2345-AA2415, AA2345-AA2375, AA2370-AA2410, AA2371-AA2502, AA2400-AA2450, AA2400-AA2425, AA2415-AA2450, AA2445-AA2500, AA2445-AA2475, AA2470-AA2490, AA2500-AA2550, AA2505-AA2540, AA2535-AA2560, AA2550-AA2600, AA2560-AA2580, AA2600-AA2650, AA2605-AA2620, AA2620-AA2650, AA2640-AA2660, AA2650-AA2700, AA2655-AA2670, AA2670-AA2700, AA2700-AA2750, AA2740-AA2760, AA2750-AA2800, AA2755-AA2780, AA2780-AA2830, AA2785-AA2810, AA2796-AA2886, AA2810-AA2825, AA2800-AA2850, AA2850-AA2900, AA2850-AA2865, AA2885-AA2905, AA2900-AA2950, AA2910-AA2930, AA2925-AA2950, AA2945-end(C terminal).

Still another aspect of the invention is a monoclonal antibody directed against an epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or

8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

Yet another aspect of the invention is a preparation of purified polyclonal antibodies directed against a polypeptide comprised of an epitope encoded within HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

Still another aspect of the invention is a polynucleotide probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or from the complement of the HCV cDNA sequence.

Yet another aspect of the invention is a kit for analyzing samples for the presence of polynucleotides from HCV comprising a polynucleotide probe containing a nucleotide sequence of about 8 or more nucleotides, wherein the nucleotide sequence is derived from HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, wherein the polynucleotide probe is in a suitable container.

Another aspect of the invention is a kit for analyzing samples for the presence of an HCV antigen comprising an antibody which reacts immunologically with an HCV antigen, wherein the antigen contains an epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

Yet another aspect of the invention is a kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide containing an HCV epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

Another aspect of the invention is a kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide expressed from HCV cDNA in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, wherein the antigenic polypeptide is present in a suitable container.

Still another aspect of the invention is a method for detecting HCV nucleic acids in a sample comprising:

(a) reacting nucleic acids of the sample with a polynucleotide probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA sequence is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, and wherein the reacting is under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample; and (b) detecting a polynucleotide duplex which contains the probe, formed in step (a).

Yet another aspect of the invention is an immunoassay for detecting an HCV antigen comprising:

(a) incubating a sample suspected of containing an HCV antigen with an antibody directed against an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-antibody complex; and (b) detecting an antibody-antigen complex formed in step (a) which contains the antibody.

Still another aspect of the invention is an immunoassay for detecting antibodies directed against an HCV antigen comprising:

(a) incubating a sample suspected of containing anti-HCV antibodies with an antigen polypeptide containing an epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-antibody complex; and detecting an antibody-antigen complex formed in step (a) which contains the antigen polypeptide.

Another aspect of the invention is a vaccine for treatment of HCV infection comprising an immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence

indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

Yet another aspect of the invention is a method for producing antibodies to HCV comprising administering to an individual an isolated immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is of the sequence present in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

Still another aspect of the invention is an antisense polynucleotide derived from HCV cDNA, wherein the HCV cDNA is that shown in Fig. 17.

Yet another aspect of the invention is a method for preparing purified fusion polypeptide C100-3 comprising:

- (a) providing a crude cell lysate containing polypeptide C100-3,
- (b) treating the crude cell lysate with an amount of acetone which causes the polypeptide to precipitate, and solubilizing the precipitated
- (c) isolatin material,
- (d) isolating the C100-3 polypeptide by anion exchange chromatography, and
- (e) further isolating the C100-3 polypeptide of step (d) by gel filtration.

25 Brief Description of the Drawings

Fig. 1 shows the sequence of the HCV cDNA in clone 12f, and the amino acids encoded therein.

Fig. 2 shows the HCV cDNA sequence in clone k9-1, and the amino acids encoded therein.

Fig. 3 shows the sequence of clone 15e, and the amino acids encoded therein.

Fig. 4 shows the nucleotide sequence of HCV cDNA in clone 13i, the amino acids encoded therein, and the sequences which overlap with clone 12f.

Fig. 5 shows the nucleotide sequence of HCV cDNA in clone 26j, the amino acids encoded therein, and the sequences which overlap clone 13i.

Fig. 6 shows the nucleotide sequence of HCV cDNA in clone CA59a, the amino acids encoded therein, and the sequences which overlap with clones 26j and K9-1.

Fig. 7 shows the nucleotide sequence of HCV cDNA in clone CA84a, the amino acids encoded therein, and the sequences which overlap with clone CA59a.

Fig. 8 shows the nucleotide sequence of HCV cDNA in clone CA156e, the amino acids encoded therein, and the sequences which overlap with CA84a.

Fig. 9 shows the nucleotide sequence of HCV cDNA in clone CA167b, the amino acids encoded therein, and the sequences which overlap CA156e.

Fig. 10 shows the nucleotide sequence of HCV cDNA in clone CA216a, the amino acids encoded therein, and the overlap with clone CA167b.

Fig. 11 shows the nucleotide sequence of HCV cDNA in clone CA290a, the amino acids encoded therein, and the overlap with clone CA216a.

Fig. 12 shows the nucleotide sequence of HCV cDNA in clone ag30a and the overlap with clone CA290a.

Fig. 13 shows the nucleotide sequence of HCV cDNA in clone CA205a, and the overlap with the HCV cDNA sequence in clone CA290a.

Fig. 14 shows the nucleotide sequence of HCV cDNA in clone 18g, and the overlap with the HCV cDNA sequence in clone ag30a.

Fig. 15 shows the nucleotide sequence of HCV cDNA in clone 16jh, the amino acids encoded therein, and the overlap of nucleotides with the HCV cDNA sequence in clone 15e.

Fig. 16 shows the ORF of HCV cDNA derived from clones pi14a, CA167b, CA156e, CA84a, CA59a, K9-1, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, and 15e.

Fig. 17 shows the sense strand of the compiled HCV cDNA sequence derived from the above-described clones and the compiled HCV cDNA sequence published in EPO Pub. No. 318,216. The clones

from which the sequence was derived are b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 33g, 39c, 35f, 19g, 26g, 15e, b5a, and 16jh. In the figure the three horizontal dashes above the sequence indicate the position of the putative initiator methionine codon; the two vertical dashes indicate the first and last nucleotides of the published sequence. Also shown in the figure is the amino acid sequence of the putative polyprotein encoded in the HCV cDNA.

Fig. 18 is a diagram of the immunological colony screening method used in antigenic mapping studies.

Fig. 19 shows the hydrophobicity profiles of polyproteins encoded in HCV and in West Nile virus.

Fig. 20 is a tracing of the hydrophilicity/hydrophobicity profile and of the antigenic index of the putative HCV polyprotein.

Fig. 21 shows the conserved co-linear peptides in HCV and Flaviviruses.

Modes for Carrying Out the Invention

15

I. Definitions

The term "hepatitis C virus" has been reserved by workers in the field for an heretofore unknown etiologic agent of NANBH. Accordingly, as used herein, "hepatitis C virus" (HCV) refers to an agent causitive of NANBH, which was formerly referred to as NANBV and/or BB-NANBV. The terms HCV, NANBV, and BB-NANBV are used interchangeably herein. As an extension of this terminology, the disease caused by HCV, formerly called NANB hepatitis (NANBH), is called hepatitis C. The terms NANBH and hepatitis C may be used interchangeably herein.

The term "HCV", as used herein, denotes a viral species of which pathogenic strains cause NANBH, and attenuated strains or defective interfering particles derived therefrom. As shown infra., the HCV genome is comprised of RNA. It is known that RNA containing viruses have relatively high rates of spontaneous mutation, i.e., reportedly on the order of 10^{-3} to 10^{-4} per incorporated nucleotide (Fields & Knipe (1986)). Therefore, there are multiple strains, which may be virulent or avirulent, within the HCV species described infra. The compositions and methods described herein, enable the propagation, identification, detection, and isolation of the various HCV strains or isolates. Moreover, the disclosure herein allows the preparation of diagnostics and vaccines for the various strains, as well as compositions and methods that have utility in screening procedures for anti-viral agents for pharmacologic use, such as agents that inhibit replication of HCV.

The information provided herein, although derived from the prototype strain or isolate of HCV, hereinafter referred to as CDC/HCV1 (also called HCV1), is sufficient to allow a viral taxonomist to identify other strains which fall within the species. The information provided herein allows the belief that HCV is a Flavi-like virus. The morphology and composition of Flavivirus particles are known, and are discussed in Brinton (1986). Generally, with respect to morphology, Flaviviruses contain a central nucleocapsid surrounded by a lipid bilayer. Virions are spherical and have a diameter of about 40-50 nm. Their cores are about 25-30 nm in diameter. Along the outer surface of the virion envelope are projections that are about 5-10 nm long with terminal knobs about 2 nm in diameter.

Different strains or isolates of HCV are expected to contain variations at the amino acid and nucleic acids compared with the prototype isolate, HCV1. Many isolates are expected to show much (i.e. more than about 40%) homology in the total amino acid sequence compared with HCV1. However, it may also be found that other less homologous HCV isolates. These would be defined as HCV strains according to various criteria such as an ORF of approximately 9,000 nucleotides to approximately 12,000 nucleotides, encoding a polyprotein similar in size to that of HCV1, an encoded polyprotein of similar hydrophobic and antigenic character to that of HCV1, and the presence of co-linear peptide sequences that are conserved with HCV1. In addition, the genome would be a positive-stranded RNA.

HCV encodes at least one epitope which is immunologically identifiable with an epitope in the HCV genome from which the cDNAs described herein are derived; preferably the epitope is contained an amino acid sequence described herein. The epitope is unique to HCV when compared to other known Flaviviruses. The uniqueness of the epitope may be determined by its immunological reactivity with anti-HCV antibodies and lack of immunological reactivity with antibodies to other Flavivirus species. Methods for determining immunological reactivity are known in the art, for example, by radioimmunoassay, by Elisa assay, by hemagglutination, and several examples of suitable techniques for assays are provided herein.

In addition to the above, the following parameters of nucleic acid homology and amino acid homology

are applicable, either alone or in combination, in identifying a strain or isolate as HCV. Since HCV strains and isolates are evolutionarily related, it is expected that the overall homology of the genomes at the nucleotide level probably will be about 40% or greater, probably about 60% or greater, and even more probably about 80% or greater; and in addition that there will be corresponding contiguous sequences of at least about 13 nucleotides. The correspondence between the putative HCV strain genomic sequence and the CDC/HCV1 cDNA sequence can be determined by techniques known in the art. For example, they can be determined by a direct comparison of the sequence information of the polynucleotide from the putative HCV, and the HCV cDNA sequence(s) described herein. For example, also, they can be determined by hybridization of the polynucleotides under conditions which form stable duplexes between homologous regions (for example, those which would be used prior to S₁ digestion), followed by digestion with single stranded specific nuclease(s), followed by size determination of the digested fragments.

Because of the evolutionary relationship of the strains or isolates of HCV, putative HCV strains or isolates are identifiable by their homology at the polypeptide level. Generally, HCV strains or isolates are expected to be more than about 40% homologous, probably more than about 70% homologous, and even more probably more than about 80% homologous, and some may even be more than about 90% homologous at the polypeptide level. The techniques for determining amino acid sequence homology are known in the art. For example, the amino acid sequence may be determined directly and compared to the sequences provided herein. Alternatively the nucleotide sequence of the genomic material of the putative HCV may be determined (usually via a cDNA intermediate), the amino acid sequence encoded therein can be determined, and the corresponding regions compared.

As used herein, a polynucleotide "derived from" a designated sequence refers to a polynucleotide sequence which is comprised of a sequence of approximately at least about 6 nucleotides, preferably at least about 8 nucleotides, more preferably at least about 10-12 nucleotides, and even more preferably at least about 15-20 nucleotides corresponding to a region of the designated nucleotide sequence. "Corresponding" means homologous to or complementary to the designated sequence. Preferably, the sequence of the region from which the polynucleotide is derived is homologous to or complementary to a sequence which is unique to an HCV genome. Whether or not a sequence is unique to the HCV genome can be determined by techniques known to those of skill in the art. For example, the sequence can be compared to sequences in databanks, e.g., Genebank, to determine whether it is present in the uninfected host or other organisms. The sequence can also be compared to the known sequences of other viral agents, including those which are known to induce hepatitis, e.g., HAV, HBV, and HDV, and to other members of the Flaviviridae. The correspondence or non-correspondence of the derived sequence to other sequences can also be determined by hybridization under the appropriate stringency conditions. Hybridization techniques for determining the complementarity of nucleic acid sequences are known in the art, and are discussed infra. See also, for example, Maniatis et al. (1982). In addition, mismatches of duplex polynucleotides formed by hybridization can be determined by known techniques, including for example, digestion with a nuclease such as S₁ that specifically digests single-stranded areas in duplex polynucleotides. Regions from which typical DNA sequences may be "derived" include but are not limited to, for example, regions encoding specific epitopes, as well as non-transcribed and/or non-translated regions.

The derived polynucleotide is not necessarily physically derived from the nucleotide sequence shown, but may be generated in any manner, including for example, chemical synthesis or DNA replication or reverse transcription or transcription. In addition, combinations of regions corresponding to that of the designated sequence may be modified in ways known in the art to be consistent with an intended use.

Similarly, a polypeptide or amino acid sequence "derived from" a designated nucleic acid sequence refers to a polypeptide having an amino acid sequence identical to that of a polypeptide encoded in the sequence, or a portion thereof wherein the portion consists of at least 3-5 amino acids, and more preferably at least 8-10 amino acids, and even more preferably at least 11-15 amino acids, or which is immunologically identifiable with a polypeptide encoded in the sequence.

A recombinant or derived polypeptide is not necessarily translated from a designated nucleic acid sequence, for example, the HCV cDNA sequences described herein, or from an HCV genome; it may be generated in any manner, including for example, chemical synthesis, or expression of a recombinant expression system, or isolation from mutated HCV. A recombinant or derived polypeptide may include one or more analogs of amino acids or unnatural amino acids in its sequence. Methods of inserting analogs of amino acids into a sequence are known in the art. It also may include one or more labels, which are known to those of skill in the art.

The term "recombinant polynucleotide" as used herein intends a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which, by virtue of its origin or manipulation which: (1) is not associated with all or a portion of a polynucleotide with which it is associated in nature, (2) is linked to a polynucleotide

other than that to which it is linked in nature, or (3) does not occur in nature.

The term "polynucleotide" as used herein refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides. This term refers only to the primary structure of the molecule. Thus, this term includes double- and single-stranded DNA, as well as double- and single stranded RNA. It also includes known types of modifications, for example, labels which are known in the art, methylation, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example proteins (including for e.g., nucleases, toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide.

The term "purified viral polynucleotide" refers to an HCV genome or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and even more preferably less than about 90% of polypeptides with which the viral polynucleotide is naturally associated. Techniques for purifying viral polynucleotides from viral particles are known in the art, and include for example, disruption of the particle with a chaotropic agent, differential extraction and separation of the polynucleotide(s) and polypeptides by ion-exchange chromatography, affinity chromatography, and sedimentation according to density.

The term "purified viral polypeptide" refers to an HCV polypeptide or fragment thereof which is essentially free, i.e., contains less than about 50%, preferably less than about 70%, and even more preferably less than about 90%, of cellular components with which the viral polypeptide is naturally associated. Techniques for purifying viral polypeptides are known in the art, and examples of these techniques are discussed infra. The term "purified viral polynucleotide" refers to an HCV genome or fragment thereof which is essentially free, i.e., contains less than about 20%, preferably less than about 50%, and even more preferably less than about 70% of polypeptides with which the viral polynucleotide is naturally associated. Techniques for purifying viral polynucleotides from viral particles are known in the art, and include for example, disruption of the particle with a chaotropic agent, and separation of the polynucleotide(s) and polypeptides by ion-exchange chromatography, affinity chromatography, and sedimentation according to density.

"Recombinant host cells", "host cells", "cells", "cell lines", "cell cultures", and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities refer to cells which can be, or have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell which has been transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation.

A "replicon" is any genetic element, e.g., a plasmid, a chromosome, a virus, a cosmid, etc. that behaves as an autonomous unit of polynucleotide replication within a cell; i.e., capable of replication under its own control.

A "vector" is a replicon in which another polynucleotide segment is attached, so as to bring about the replication and/or expression of the attached segment.

"Control sequence" refers to polynucleotide sequences which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and terminators; in eukaryotes, generally, such control sequences include promoters, terminators and, in some instances, enhancers. The term "control sequences" is intended to include, at a minimum, all components whose presence is necessary for expression, and may also include additional components whose presence is advantageous, for example, leader sequences.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

An "open reading frame" (ORF) is a region of a polynucleotide sequence which encodes a polypeptide; this region may represent a portion of a coding sequence or a total coding sequence.

A "coding sequence" is a polynucleotide sequence which is transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a translation start codon at the 5'-terminus and a translation stop

codon at the 3'-terminus. A coding sequence can include, but is not limited to mRNA, cDNA, and recombinant polynucleotide sequences.

"Immunologically identifiable with/as" refers to the presence of epitope(s) and polypeptides(s) which are also present in the designated polypeptide(s), usually HCV proteins. Immunological identity may be determined by antibody binding and/or competition in binding; these techniques are known to those of average skill in the art, and are also illustrated infra.

As used herein, "epitope" refers to an antigenic determinant of a polypeptide; an epitope could comprise 3 amino acids in a spatial conformation which is unique to the epitope, generally an epitope consists of at least 5 such amino acids, and more usually, consists of at least 8-10 such amino acids.

Methods of determining the spatial conformation of amino acids are known in the art, and include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance.

A polypeptide is "immunologically reactive" with an antibody when it binds to an antibody due to antibody recognition of a specific epitope contained within the polypeptide. Immunological reactivity may be determined by antibody binding, more particularly by the kinetics of antibody binding, and/or by competition in binding using as competitor(s) a known polypeptide(s) containing an epitope against which the antibody is directed. The techniques for determining whether a polypeptide is immunologically reactive with an antibody are known in the art.

As used herein, the term "immunogenic polypeptide" is a polypeptide that elicits a cellular and/or humoral response, whether alone or linked to a carrier in the presence or absence of an adjuvant.

The term "polypeptide" refers to a polymer of amino acids and does not refer to a specific length of the product; thus, peptides, oligopeptides, and proteins are included within the definition of polypeptide. This term also does not refer to or exclude post-expression modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages, as well as other modifications known in the art, both naturally occurring and non-naturally occurring.

"Transformation", as used herein, refers to the insertion of an exogenous polynucleotide into a host cell, irrespective of the method used for the insertion, for example, direct uptake, transduction, f-mating or electroporation. The exogenous polynucleotide may be maintained as a non-integrated vector, for example, a plasmid, or alternatively, may be integrated into the host genome.

"Treatment" as used herein refers to prophylaxis and/or therapy.

An "individual", as used herein, refers to vertebrates, particularly members of the mammalian species, and includes but is not limited to domestic animals, sports animals, and primates, including humans.

As used herein, the "sense strand" of a nucleic acid contains the sequence that has sequence homology to that of mRNA. The "anti-sense strand" contains a sequence which is complementary to that of the "sense strand".

As used herein, a "positive stranded genome" of a virus is one in which the genome, whether RNA or DNA, is single-stranded and which encodes a viral polypeptide(s). Examples of positive stranded RNA viruses include Togaviridae, Coronaviridae, Retroviridae, Picornaviridae, and Caliciviridae. Included also, are the Flaviviridae, which were formerly classified as Togaviridae. See Fields & Knipe (1986).

As used herein, "antibody-containing body component" refers to a component of an individual's body which is a source of the antibodies of interest. Antibody containing body components are known in the art, and include but are not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, white blood cells, and myelomas.

As used herein, "purified HCV" refers to a preparation of HCV which has been isolated from the cellular constituents with which the virus is normally associated, and from other types of viruses which may be present in the infected tissue. The techniques for isolating viruses are known to those of skill in the art, and include, for example, centrifugation and affinity chromatography; a method of preparing purified HCV is discussed infra.

The term "HCV particles" as used herein include entire virion as well as particles which are intermediates in virion formation. HCV particles generally have one or more HCV proteins associated with the HCV nucleic acid.

As used herein, the term "probe" refers to a polynucleotide which forms a hybrid structure with a sequence in a target region, due to complementarity of at least one sequence in the probe with a sequence in the target region. The probe, however, does not contain a sequence complementary to sequence(s) used to prime the polymerase chain reaction.

As used herein, the term "target region" refers to a region of the nucleic acid which is to be amplified

and/or detected:

As used herein, the term "viral RNA", which includes HCV RNA, refers to RNA from the viral genome, fragments thereof, transcripts thereof, and mutant sequences derived therefrom.

- As used herein, a "biological sample" refers to a sample of tissue or fluid isolated from an individual, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs, and also samples of *in vitro* cell culture constituents (including but not limited to conditioned medium resulting from the growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components).

10

II. Description of the Invention

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. See e.g., Maniatis, Fitch & Sambrook, MOLECULAR CLONING: A LABORATORY MANUAL (1982); DNA CLONING, VOLUMES I AND II (D.N. Glover ed. 1985); OLIGONUCLEOTIDE SYNTHESIS (M.J. Gait ed, 1984); NUCLEIC ACID HYBRIDIZATION (B.D. Hames & S.J. Higgins eds. 1984); TRANSCRIPTION AND TRANSLATION (B.D. Hames & S.J. Higgins eds. 1984); ANIMAL CELL CULTURE (R.I. Freshney ed. 1986); IMMOBILIZED CELLS AND ENZYMES (IRL Press, 1986); B. Perbal, A PRACTICAL GUIDE TO MOLECULAR CLONING (1984); the series, METHODS IN ENZYMOLOGY (Academic Press, Inc.); GENE TRANSFER VECTORS FOR MAMMALIAN CELLS (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory), Methods in Enzymology Vol. 154 and Vol. 155 (Wu and Grossman, and Wu, eds., respectively), Mayer and Walker, eds. (1987), IMMUNOCHEMICAL METHODS IN CELL AND MOLECULAR BIOLOGY (Academic Press, London), Scopes, (1987), PROTEIN PURIFICATION: PRINCIPLES AND PRACTICE, Second Edition (Springer-Verlag, N.Y.), and HANDBOOK OF EXPERIMENTAL IMMUNOLOGY, VOLUMES I-IV (D.M. Weir and C. C. Blackwell eds 1986). All patents, patent applications, and publications mentioned herein, both *supra* and *infra*, are hereby incorporated herein by reference.

30 The useful materials and processes of the present invention are made possible by the provision of a family of nucleotide sequences isolated from cDNA libraries which contain HCV cDNA sequences. These cDNA libraries were derived from nucleic acid sequences present in the plasma of an HCV-infected chimpanzee. The construction of one of these libraries, the "c" library (ATCC No. 40394), was reported in EPO Pub. No. 318,216. Several of the clones containing HCV cDNA reported herein were obtained from the "c" library. Although other clones reported herein were obtained from other HCV cDNA libraries, the presence of clones containing the sequences in the "c" library was confirmed. As discussed in EPO Pub. No. 318,216, the family of HCV cDNA sequences isolated from the "c" library are not of human or chimpanzee origin, and show no significant homology to sequences contained within the HBV genome.

35 The availability of the HCV cDNAs described herein permits the construction of polynucleotide probes which are reagents useful for detecting viral polynucleotides in biological samples, including donated blood. For example, from the sequences it is possible to synthesize DNA oligomers of about 8-10 nucleotides, or larger, which are useful as hybridization probes to detect the presence of HCV RNA in, for example, donated blood, sera of subjects suspected of harboring the virus, or cell culture systems in which the virus is replicating. In addition, the cDNA sequences also allow the design and production of HCV specific 40 polypeptides which are useful as diagnostic reagents for the presence of antibodies raised during HCV infection. Antibodies to purified polypeptides derived from the cDNAs may also be used to detect viral 45 antigens in biological samples, including, for example, donated blood samples, sera from patients with NANBH, and in tissue culture systems being used for HCV replication. Moreover, the immunogenic polypeptides disclosed herein, which are encoded in portions of the ORF of HCV cDNA shown in Fig. 17, 50 are also useful for HCV screening, diagnosis, and treatment, and for raising antibodies which are also useful for these purposes.

In addition, the novel cDNA sequences described herein enable further characterization of the HCV genome. Polynucleotide probes and primers derived from these sequences may be used to amplify 55 sequences present in cDNA libraries, and/or to screen cDNA libraries for additional overlapping cDNA sequences, which, in turn, may be used to obtain more overlapping sequences. As indicated *infra*, and in EPO Pub. No. 318,216, the genome of HCV appears to be RNA comprised primarily of a large open reading frame (ORF) which encodes a large polyprotein.

The HCV cDNA sequences provided herein, the polypeptides derived from these sequences, and the

immunogenic polypeptides described herein, as well as antibodies directed against these polypeptides are also useful in the isolation and identification of the blood-borne NABV (BB-NANBV) agent(s). For example, antibodies directed against HCV epitopes contained in polypeptides derived from the cDNAs may be used in processes based upon affinity chromatography to isolate the virus. Alternatively, the antibodies may be 5 used to identify viral particles isolated by other techniques. The viral antigens and the genomic material within the isolated viral particles may then be further characterized.

In addition to the above, the information provided infra allows the identification of additional HCV strains or isolates. The isolation and characterization of the additional HCV strains or isolates may be accomplished by isolating the nucleic acids from body components which contain viral particles and/or viral RNA, creating 10 cDNA libraries using polynucleotide probes based on the HCV cDNA probes described infra., screening the libraries for clones containing HCV cDNA sequences described infra., and comparing the HCV cDNAs from the new isolates with the cDNAs described infra. The polypeptides encoded therein, or in the viral genome, may be monitored for immunological cross-reactivity utilizing the polypeptides and antibodies described supra. Strains or isolates which fit within the parameters of HCV, as described in the Definitions section, 15 supra., are readily identifiable. Other methods for identifying HCV strains will be obvious to those of skill in the art, based upon the information provided herein.

Isolation of the HCV cDNA Sequences

20 The novel HCV cDNA sequences described infra. extend the sequence of the cDNA to the HCV genome reported in EPO Pub. No. 318,216. The sequences which are present in clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, and CA59a lie upstream of the reported sequence, and when compiled, yield nucleotides nos. -319 to 1348 of the composite HCV cDNA sequence. 25 (The negative number on a nucleotide indicates its distance upstream of the nucleotide which starts the putative initiator MET codon.) The sequences which are present in clones b5a and 16jh lie downstream of the reported sequence, and yield nucleotides nos. 8659 to 8866 of the composite sequence. The composite HCV cDNA sequence which includes the sequences in the aforementioned clones, is shown in Fig. 17.

The novel HCV cDNAs described herein were isolated from a number of HCV cDNA libraries, including 30 the "c" library present in lambda gt11 (ATCC No. 40394). The HCV cDNA libraries were constructed using pooled serum from a chimpanzee with chronic HCV infection and containing a high titer of the virus, i.e., at least 10^6 chimp infectious doses/ml (CID/ml). The pooled serum was used to isolate viral particles; nucleic acids isolated from these particles was used as the template in the construction of cDNA libraries to the viral genome. The procedures for isolation of putative HCV particles and for constructing the "c" HCV cDNA 35 library is described in EPO Pub. No. 318,216. Other methods for constructing HCV cDNA libraries are known in the art, and some of these methods are described infra., in the Examples. Isolation of the sequences was by screening the libraries using synthetic polynucleotide probes, the sequences of which were derived from the 5'-region and the 3'-region of the known HCV cDNA sequence. The description of the method to retrieve the cDNA sequences is mostly of historical interest. The resultant sequences (and 40 their complements) are provided herein, and the sequences, or any portion thereof, could be prepared using synthetic methods, or by a combination of synthetic methods with retrieval of partial sequences using methods similar to those described herein.

45 Preparation of Viral Polypeptides and Fragments

The availability of HCV cDNA sequences, or nucleotide sequences derived therefrom (including segments and modifications of the sequence), permits the construction of expression vectors encoding antigenically active regions of the polypeptide encoded in either strand. These antigenically active regions 50 may be derived from coat or envelope antigens or from core antigens, or from antigens which are non-structural including, for example, polynucleotide binding proteins, polynucleotide polymerase(s), and other viral proteins required for the replication and/or assembly of the virus particle. Fragments encoding the desired polypeptides are derived from the cDNA clones using conventional restriction digestion or by synthetic methods, and are ligated into vectors which may, for example, contain portions of fusion 55 sequences such as beta-galactosidase or superoxide dismutase (SOD), preferably SOD. Methods and vectors which are useful for the production of polypeptides which contain fusion sequences of SOD are described in European Patent Office Publication number 0196056, published October 1, 1986. Vectors for the expression of fusion polypeptides of SOD and HCV polypeptides encoded in a number of HCV clones

are described infra., in the Examples. Any desired portion of the HCV cDNA containing an open reading frame, in either sense strand, can be obtained as a recombinant polypeptide, such as a mature or fusion protein; alternatively, a polypeptide encoded in the cDNA can be provided by chemical synthesis.

The DNA encoding the desired polypeptide, whether in fused or mature form, and whether or not containing a signal sequence to permit secretion, may be ligated into expression vectors suitable for any convenient host. Both eukaryotic and prokaryotic host systems are presently used in forming recombinant polypeptides, and a summary of some of the more common control systems and host cell lines is given infra. The polypeptide is then isolated from lysed cells or from the culture medium and purified to the extent needed for its intended use. Purification may be by techniques known in the art, for example, differential extraction, salt fractionation, chromatography on ion exchange resins, affinity chromatography, centrifugation, and the like. See, for example, *Methods in Enzymology* for a variety of methods for purifying proteins. Such polypeptides can be used as diagnostics, or those which give rise to neutralizing antibodies may be formulated into vaccines. Antibodies raised against these polypeptides can also be used as diagnostics, or for passive immunotherapy. In addition, as discussed infra., antibodies to these polypeptides are useful for isolating and identifying HCV particles.

Preparation of Antigenic Polypeptides and Conjugation with Carrier

An antigenic region of a polypeptide is generally relatively small—typically 8 to 10 amino acids or less in length. Fragments of as few as 5 amino acids may characterize an antigenic region. These segments may correspond to regions of HCV antigen. Accordingly, using the cDNAs of HCV as a basis, DNAs encoding short segments of HCV polypeptides can be expressed recombinantly either as fusion proteins, or as isolated polypeptides. In addition, short amino acid sequences can be conveniently obtained by chemical synthesis. In instances wherein the synthesized polypeptide is correctly configured so as to provide the correct epitope, but is too small to be immunogenic, the polypeptide may be linked to a suitable carrier.

A number of techniques for obtaining such linkage are known in the art, including the formation of disulfide linkages using N-succinimidyl-3-(2-pyridylthio)propionate (SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) obtained from Pierce Company, Rockford, Illinois, (if the peptide lacks a sulphydryl group, this can be provided by addition of a cysteine residue.) These reagents create a disulfide linkage between themselves and peptide cysteine residues on one protein and an amide linkage through the epsilon-amino on a lysine, or other free amino group in the other. A variety of such disulfide/amide-forming agents are known. See, for example, *Immun. Rev.* (1982) 62:185. Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of these thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocaproic acid, 2-bromoacetic acid, 2-iodoacetic acid, 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid, and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Additional methods of coupling antigens employs the rotavirus/"binding peptide" system described in EPO Pub. No. 259,149, the disclosure of which is incorporated herein by reference. The foregoing list is not meant to be exhaustive, and modifications of the named compounds can clearly be used.

Any carrier may be used which does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins; polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles. Especially useful protein substrates are serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those skilled in the art.

In addition to full-length viral proteins, polypeptides comprising truncated HCV amino acid sequences encoding at least one viral epitope are useful immunological reagents. For example, polypeptides comprising such truncated sequences can be used as reagents in an immunoassay. These polypeptides also are candidate subunit antigens in compositions for antiserum production or vaccines. While these truncated sequences can be produced by various known treatments of native viral protein, it is generally preferred to make synthetic or recombinant polypeptides comprising an HCV sequence. Polypeptides comprising these truncated HCV sequences can be made up entirely of HCV sequences (one or more epitopes, either contiguous or noncontiguous), or HCV sequences and heterologous sequences in a fusion protein. Useful heterologous sequences include sequences that provide for secretion from a recombinant host, enhance the immunological reactivity of the HCV epitope(s), or facilitate the coupling of the polypeptide to an immunoassay support or a vaccine carrier. See, e.g., EPO Pub. No. 116,201; U.S. Pat. No. 4,722,840; EPO

Pub. No. 259,149; U.S. Pat. No. 4,629,783, the disclosures of which are incorporated herein by reference.

The size of polypeptides comprising the truncated HCV sequences can vary widely, the minimum size being a sequence of sufficient size to provide an HCV epitope, while the maximum size is not critical. For convenience, the maximum size usually is not substantially greater than that required to provide the desired HCV epitopes and function(s) of the heterologous sequence, if any. Typically, the truncated HCV amino acid sequence will range from about 5 to about 100 amino acids in length. More typically, however, the HCV sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 30 amino acids. It is usually desirable to select HCV sequences of at least about 10, 12 or 15 amino acids, up to a maximum of about 20 or 25 amino acids.

- Truncated HCV amino acid sequences comprising epitopes can be identified in a number of ways. For example, the entire viral protein sequence can be screened by preparing a series of short peptides that together span the entire protein sequence. An example of antigenic screening of the regions of the HCV polyprotein is shown infra. In addition, by starting with, for example, 100mer polypeptides, it would be routine to test each polypeptide for the presence of epitope(s) showing a desired reactivity, and then testing progressively smaller and overlapping fragments from an identified 100mer to map the epitope of interest. Screening such peptides in an immunoassay is within the skill of the art. It is also known to carry out a computer analysis of a protein sequence to identify potential epitopes, and then prepare oligopeptides comprising the identified regions for screening. Such a computer analysis of the HCV amino acid sequence is shown in Fig. 20, where the hydrophilic/hydrophobic character is displayed above the antigen index. The amino acids are numbered from the starting MET (position 1) as shown in Fig. 17. It is appreciated by those of skill in the art that such computer analysis of antigenicity does not always identify an epitope that actually exists, and can also incorrectly identify a region of the protein as containing an epitope. Examples of HCV amino acid sequences that may be useful, which are expressed from expression vectors comprised of clones 5-1-1, 81, CA74a, 35f, 279a, C36, C33b, CA290a, C8f, C12f, 14c, 15e, C25c, C33c, C33f, 33g, C39c, C40b, CA167b are described infra. Other examples of HCV amino acid sequences that may be useful as described herein are set forth below. It is to be understood that these peptides do not necessarily precisely map one epitope, and may also contain HCV sequence that is not immunogenic. These non-immunogenic portions of the sequence can be defined as described above using conventional techniques and deleted from the described sequences. Further, additional truncated HCV amino acid sequences that comprise an epitope or are immunogenic can be identified as described above. The following sequences are given by amino acid number (i.e., "AA_n") where n is the amino acid number as shown in Fig. 17:
- AA1-AA25; AA1-AA50; AA1-AA84; AA9-AA177; AA1-AA10; AA5-AA20; AA20-AA25; AA35-AA45; AA50-AA100; AA40-AA90; AA45-AA65; AA65-AA75; AA80-90; AA99-AA120; AA95-AA110; AA105-AA120; AA100-AA150; AA150-AA200; AA155-AA170; AA190-AA210; AA200-AA250; AA220-AA240; AA245-AA265; AA250-AA300; AA290-AA330; AA290-305; AA300-AA350; AA310-AA330; AA350-AA400; AA380-AA395; AA405-AA495; AA400-AA450; AA405-AA415; AA415-AA425; AA425-AA435; AA437-AA582; AA450-AA500; AA440-AA460; AA460-AA470; AA475-AA495; AA500-AA550; AA511-AA690; AA515-AA550; AA550-AA600; AA550-AA625; AA575-AA605; AA585-AA600; AA600-AA650; AA600-AA625; AA635-AA665; AA650-AA700; AA645-AA680; AA700-AA750; AA700-AA725; AA700-AA750; AA725-AA775; AA770-AA790; AA750-AA800; AA800-AA815; AA825-AA850; AA850-AA875; AA800-AA850; AA920-AA990; AA850-AA900; AA920-AA945; AA940-AA965; AA970-AA990; AA950-AA1000; AA1000-AA1060; AA1000-AA1025; AA1000-AA1050; AA1025-AA1040; AA1040-AA1055; AA1075-AA1175; AA1050-AA1200; AA1070-AA1100; AA1100-AA1130; AA1140-AA1165; AA1192-AA1457; AA1195-AA1250; AA1200-AA1225; AA1225-AA1250; AA1250-AA1300; AA1260-AA1310; AA1260-AA1280; AA1266-AA1428; AA1300-AA1350; AA1290-AA1310; AA1310-AA1340; AA1345-AA1405; AA1345-AA1365; AA1350-AA1400; AA1365-AA1380; AA1380-AA1405; AA1400-AA1450; AA1450-AA1500; AA1460-AA1475; AA1475-AA1515; AA1475-AA1500; AA1500-AA1550; AA1500-AA1515; AA1515-AA1550; AA1550-AA1600; AA1545-AA1560; AA1569-AA1931; AA1570-AA1590; AA1595-AA1610; AA1590-AA1650; AA1610-AA1645; AA1650-AA1690; AA1685-AA1770; AA1689-AA1805; AA1690-AA1720; AA1694-AA1735; AA1720-AA1745; AA1745-AA1770; AA1750-AA1800; AA1775-AA1810; AA1795-AA1850; AA1850-AA1900; AA1900-AA1950; AA1900-AA1920; AA1916-AA2021; AA1920-AA1940; AA1949-AA2124; AA1950-AA2000; AA1950-AA1985; AA1980-AA2000; AA2000-AA2050; AA2005-AA2025; AA2020-AA2045; AA2045-AA2100; AA2045-AA2070; AA2054-AA2223; AA2070-AA2100; AA2100-AA2150; AA2150-AA2200; AA2200-AA2250; AA2200-AA2325; AA2250-AA2330; AA2255-AA2270; AA2265-AA2280; AA2280-AA2290; AA2287-AA2385; AA2300-AA2350; AA2290-AA2310; AA2310-AA2330; AA2330-AA2350; AA2350-AA2400; AA2348-AA2464; AA2345-AA2415; AA2345-AA2375; AA2370-AA2410; AA2371-AA2502; AA2400-AA2450; AA2400-AA2425; AA2415-AA2450; AA2445-AA2500; AA2445-AA2475; AA2470-AA2490; AA2500-AA2550; AA2505-AA2540; AA2535-AA2560; AA2550-AA2600; AA2560-AA2580; AA2600-AA2650; AA2605-AA2620; AA2620-AA2650; AA2640-AA2660; AA2650-AA2700; AA2655-AA2670; AA2670-AA2700; AA2700-AA2750; AA2740-

AA2760; AA2750-AA2800; AA2755-AA2780; AA2780-AA2830; AA2785-AA2810; AA2796-AA2886; AA2810-AA2825; AA2800-AA2850; AA2850-AA2900; AA2850-AA2865; AA2885-AA2905; AA2900-AA2950; AA2910-AA2930; AA2925-AA2950; AA2945-end(C terminal).

The above HCV amino acid sequences can be prepared as discrete peptides or incorporated into a larger polypeptide, and may find use as described herein. Additional polypeptides comprising truncated HCV sequences are described in the examples.

The observed relationship of the putative polyproteins of HCV and the Flaviviruses allows some prediction of the putative domains of the HCV "non-structural" (NS) proteins. The locations of the individual NS proteins in the putative Flavivirus precursor polyprotein are fairly well-known. Moreover, these also coincide with observed gross fluctuations in the hydrophobicity profile of the polyprotein. It is established that NS5 of Flaviviruses encodes the virion polymerase, and that NS1 corresponds with a complement fixation antigen which has been shown to be an effective vaccine in animals. Recently, it has been shown that a flaviviral protease function resides in NS3. Due to the observed similarities between HCV and the Flaviviruses, described infra., deductions concerning the approximate locations of the corresponding protein domains and functions in the HCV polyprotein are possible. The expression of polypeptides containing these domains in a variety of recombinant host cells, including, for example, bacteria, yeast, insect, and vertebrate cells, should give rise to important immunological reagents which can be used for diagnosis, detection, and vaccines.

Although the non-structural protein regions of the putative polyproteins of the HCV isolate described herein and of Flaviviruses appear to have some similarity, there is less similarity between the putative structural regions which are towards the N-terminus. In this region, there is a greater divergence in sequence, and in addition, the hydrophobic profile of the two regions show less similarity. This "divergence" begins in the N-terminal region of the putative NS1 domain in HCV, and extends to the presumed N-terminus. Nevertheless, it may still be possible to predict the approximate locations of the putative nucleocapsid (N-terminal basic domain) and E (generally hydrophobic) domains within the HCV polyprotein. In the Examples the predictions are based on the changes observed in the hydrophobic profile of the HCV polyprotein, and on a knowledge of the location and character of the flaviviral proteins. From these predictions it may be possible to identify approximate regions of the HCV polyprotein that could correspond with useful immunological reagents. For example, the E and NS1 proteins of Flaviviruses are known to have efficacy as protective vaccines. These regions, as well as some which are shown to be antigenic in the HCV isolate described herein, for example those within putative NS3, C, and NS5, etc., should also provide diagnostic reagents. Moreover, the location and expression of viral-encoded enzymes may also allow the evaluation of anti-viral enzyme inhibitors, i.e., for example, inhibitors which prevent enzyme activity by virtue of an interaction with the enzyme itself, or substances which may prevent expression of the enzyme, (for example, anti-sense RNA, or other drugs which interfere with expression).

Preparation of Hybrid Particle Immunogens Containing HCV Epitopes

The immunogenicity of the epitopes of HCV may also be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. Constructs wherein the NANBV epitope is linked directly to the particle-forming protein coding sequences produce hybrids which are immunogenic with respect to the HCV epitope. In addition, all of the vectors prepared include epitopes specific to HBV, having various degrees of immunogenicity, such as, for example, the pre-S peptide. Thus, particles constructed from particle forming protein which include HCV sequences are immunogenic with respect to HCV and HBV.

Hepatitis surface antigen (HBsAg) has been shown to be formed and assembled into particles in *S. cerevisiae* (Valenzuela et al. (1982)), as well as in, for example, mammalian cells (Valenzuela, P., et al. (1984)). The formation of such particles has been shown to enhance the immunogenicity of the monomer subunit. The constructs may also include the immunodominant epitope of HBsAg, comprising the 55 amino acids of the presurface (pre-S) region. Neurath et al. (1984). Constructs of the pre-S-HBsAg particle expressible in yeast are disclosed in EPO 174,444, published March 19, 1986; hybrids including heterologous viral sequences for yeast expression are disclosed in EPO 175,261, published March 26, 1986. These constructs may also be expressed in mammalian cells such as Chinese hamster ovary (CHO) cells using an SV40-dihydrofolate reductase vector (Michelle et al. (1984)).

In addition, portions of the particle-forming protein coding sequence may be replaced with codons encoding an HCV epitope. In this replacement, regions which are not required to mediate the aggregation of the units to form immunogenic particles in yeast or mammals can be deleted, thus eliminating additional

HBV antigenic sites from competition with the HCV epitope.

Preparation of Vaccines

5 Vaccines may be prepared from one or more immunogenic polypeptides derived from HCV cDNA, including the cDNA sequences described in the Examples. The observed homology between HCV and Flaviviruses provides information concerning the polypeptides which may be most effective as vaccines, as well as the regions of the genome in which they are encoded. The general structure of the Flavivirus genome is discussed in Rice et al (1986). The flavivirus genomic RNA is believed to be the only virus-specific mRNA species, and it is translated into the three viral structural proteins, i.e., C, M, and E, as well as two large nonstructural proteins, NS4 and NS5, and a complex set of smaller nonstructural proteins. It is known that major neutralizing epitopes for Flaviviruses reside in the E (envelope) protein (Roehrig (1986)). Thus, vaccines may be comprised of recombinant polypeptides containing epitopes of HCV E. These polypeptides may be expressed in bacteria, yeast, or mammalian cells, or alternatively may be isolated from viral preparations. It is also anticipated that the other structural proteins may also contain epitopes which give rise to protective anti-HCV antibodies. Thus, polypeptides containing the epitopes of E, C, and M may also be used, whether singly or in combination, in HCV vaccines.

20 In addition to the above, it has been shown that immunization with NS1 (nonstructural protein 1), results in protection against yellow fever (Schlesinger et al (1986)). This is true even though the immunization does not give rise to neutralizing antibodies. Thus, particularly since this protein appears to be highly conserved among Flaviviruses, it is likely that HCV NS1 will also be protective against HCV infection. Moreover, it also shows that nonstructural proteins may provide protection against viral pathogenicity, even if they do not cause the production of neutralizing antibodies.

25 The information provided in the Examples concerning the immunogenicity of the polypeptides expressed from cloned HCV cDNAs which span the various regions of the HCV ORF also allows predictions concerning their use in vaccines.

30 In view of the above, multivalent vaccines against HCV may be comprised of one or more epitopes from one or more structural proteins, and/or one or more epitopes from one or more nonstructural proteins. These vaccines may be comprised of, for example, recombinant HCV polypeptides and/or polypeptides isolated from the virions. In particular, vaccines are contemplated comprising one or more of the following HCV proteins, or subunit antigens derived therefrom: E, NS1, C, NS2, NS3, NS4 and NS5. Particularly preferred are vaccines comprising E and/or NS1, or subunits thereof.

35 The preparation of vaccines which contain an immunogenic polypeptide(s) as active ingredients, is known to one skilled in the art. Typically, such vaccines are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified, or the protein encapsulated in liposomes. The active immunogenic ingredients are often mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the vaccine may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. Examples of adjuvants which may be effective include but are not limited to: aluminum hydroxide, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetyl-muramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against an immunogenic polypeptide containing an HCV antigenic sequence resulting from administration of this polypeptide in vaccines which are also comprised of the various adjuvants.

40 The vaccines are conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the

like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25%-70%.

The proteins may be formulated into the vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

10

Dosage and Administration of Vaccines

The vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is generally in the range of 5 micrograms to 250 micrograms of antigen per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgment of the practitioner and may be peculiar to each subject.

20 The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary course of vaccination may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and/or reinforce the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at least in part, be determined by the need of the individual 25 and be dependent upon the judgment of the practitioner.

In addition, the vaccine containing the immunogenic HCV antigen(s) may be administered in conjunction with other immunoregulatory agents, for example, immune globulins.

30 Preparation of Antibodies Against HCV Epitopes

The immunogenic polypeptides prepared as described above are used to produce antibodies, both polyclonal and monoclonal. If polyclonal antibodies are desired, a selected mammal (e.g., mouse, rabbit, goat, horse, etc.) is immunized with an immunogenic polypeptide bearing an HCV epitope(s). Serum from the immunized animal is collected and treated according to known procedures. If serum containing polyclonal antibodies to an HCV epitope contains antibodies to other antigens, the polyclonal antibodies can be purified by immunoaffinity chromatography. Techniques for producing and processing polyclonal antisera are known in the art, see for example, Mayer and Walker (1987).

Alternatively, polyclonal antibodies may be isolated from a mammal which has been previously infected 40 with HCV. An example of a method for purifying antibodies to HCV epitopes from serum from an infected individual, based upon affinity chromatography and utilizing a fusion polypeptide of SOD and a polypeptide encoded within cDNA clone 5-1-1, is presented in EPO Pub. No. 318,216.

Monoclonal antibodies directed against HCV epitopes can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies by hybridomas is well known. Immortal 45 antibody-producing cell lines can be created by cell fusion, and also by other techniques such as direct transformation of B lymphocytes with oncogenic DNA, or transfection with Epstein-Barr virus. See, e.g., M. Schreier et al. (1980); Hammerling et al. (1981); Kennett et al. (1980); see also, U.S. Patent Nos. 4,341,761; 4,399,121; 4,427,783; 4,444,887; 4,466,917; 4,472,500; 4,491,632; and 4,493,890. Panels of monoclonal antibodies produced against HCV epitopes can be screened for various properties; i.e., for isotype, epitope 50 affinity, etc.

Antibodies, both monoclonal and polyclonal, which are directed against HCV epitopes are particularly useful in diagnosis, and those which are neutralizing are useful in passive immunotherapy. Monoclonal antibodies, in particular, may be used to raise anti-idiotype antibodies.

Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. See, for example, Nisonoff, A., et al. (1981) and Dreesman et al. (1985).

Techniques for raising anti-idiotype antibodies are known in the art. See, for example, Grzych (1985), MacNamara et al. (1984), and Uytdehaag et al. (1985). These anti-idiotype antibodies may also be useful for

treatment and/or diagnosis of NANBH, as well as for an elucidation of the immunogenic regions of HCV antigens.

It would also be recognized by one of ordinary skill in the art that a variety of types of antibodies directed against HCV epitopes may be produced. As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one antibody combining site. An "antibody combining site" or "binding domain" is formed from the folding of variable domains of an antibody molecule(s) to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows an immunological reaction with the antigen. An antibody combining site may be formed from a heavy and/or a light chain domain (VH and VL, respectively), which form hypervariable loops which contribute to antigen binding. The term "antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, altered antibodies, univalent antibodies, the Fab proteins, and single domain antibodies.

A "single domain antibody" (dAb) is an antibody which is comprised of an VH domain, which reacts immunologically with a designated antigen. A dAB does not contain a VL domain, but may contain other antigen binding domains known to exist in antibodies, for example, the kappa and lambda domains. Methods for preparing dABs are known in the art. See, for example, Ward et al. (1989).

Antibodies may also be comprised of VH and VL domains, as well as other known antigen binding domains. Examples of these types of antibodies and methods for their preparation are known in the art (see, e.g., U.S. Patent No. 4,816,467, which is incorporated herein by reference), and include the following. For example, "vertebrate antibodies" refers to antibodies which are tetramers or aggregates thereof, comprising light and heavy chains which are usually aggregated in a "Y" configuration and which may or may not have covalent linkages between the chains. In vertebrate antibodies, the amino acid sequences of all the chains of a particular antibody are homologous with the chains found in one antibody produced by the lymphocyte which produces that antibody *in situ*, or *in vitro* (for example, in hybridomas). Vertebrate antibodies typically include native antibodies, for example, purified polyclonal antibodies and monoclonal antibodies. Examples of the methods for the preparation of these antibodies are described infra.

"Hybrid antibodies" are antibodies wherein one pair of heavy and light chains is homologous to those in a first antibody, while the other pair of heavy and light chains is homologous to those in a different second antibody. Typically, each of these two pairs will bind different epitopes, particularly on different antigens. This results in the property of "divalence", i.e., the ability to bind two antigens simultaneously. Such hybrids may also be formed using chimeric chains, as set forth below.

"Chimeric antibodies", are antibodies in which the heavy and/or light chains are fusion proteins. Typically the constant domain of the chains is from one particular species and/or class, and the variable domains are from a different species and/or class. Also included is any antibody in which either or both of the heavy or light chains are composed of combinations of sequences mimicking the sequences in antibodies of different sources, whether these sources be differing classes, or different species of origin, and whether or not the fusion point is at the variable/constant boundary. Thus, it is possible to produce antibodies in which neither the constant nor the variable region mimic known antibody sequences. It then becomes possible, for example, to construct antibodies whose variable region has a higher specific affinity for a particular antigen, or whose constant region can elicit enhanced complement fixation, or to make other improvements in properties possessed by a particular constant region.

Another example is "altered antibodies", which refers to antibodies in which the naturally occurring amino acid sequence in a vertebrate antibody has been varied. Utilizing recombinant DNA techniques, antibodies can be redesigned to obtain desired characteristics. The possible variations are many, and range from the changing of one or more amino acids to the complete redesign of a region, for example, the constant region. Changes in the constant region, in general, to attain desired cellular process characteristics, e.g., changes in complement fixation, interaction with membranes, and other effector functions. Changes in the variable region may be made to alter antigen binding characteristics. The antibody may also be engineered to aid the specific delivery of a molecule or substance to a specific cell or tissue site. The desired alterations may be made by known techniques in molecular biology, e.g., recombinant techniques, site directed mutagenesis, etc.

Yet another example are "univalent antibodies", which are aggregates comprised of a heavy chain/light chain dimer bound to the Fc (i.e., constant) region of a second heavy chain. This type of antibody escapes antigenic modulation. See, e.g., Glennie et al. (1982).

Included also within the definition of antibodies are "Fab" fragments of antibodies. The "Fab" region refers to those portions of the heavy and light chains which are roughly equivalent, or analogous, to the sequences which comprise the branch portion of the heavy and light chains, and which have been shown to exhibit immunological binding to a specified antigen, but which lack the effector Fc portion. "Fab" includes

aggregates of one heavy and one light chain (commonly known as Fab'), as well as tetramers containing the 2H and 2L chains (referred to as F(ab)₂), which are capable of selectively reacting with a designated antigen or antigen family. "Fab" antibodies may be divided into subsets analogous to those described above, i.e., "vertebrate Fab", "hybrid Fab", "chimeric Fab", and "altered Fab". Methods of producing "Fab" fragments of antibodies are known within the art and include, for example, proteolysis, and synthesis by recombinant techniques.

II.H. Diagnostic Oligonucleotide Probes and Kits

Using the disclosed portions of the isolated HCV cDNAs as a basis, oligomers of approximately 8 nucleotides or more can be prepared, either by excision or synthetically, which hybridize with the HCV genome and are useful in identification of the viral agent(s), further characterization of the viral genome(s), as well as in detection of the virus(es) in diseased individuals. The probes for HCV polynucleotides (natural or derived) are a length which allows the detection of unique viral sequences by hybridization. While 6-8 nucleotides may be a workable length, sequences of 10-12 nucleotides are preferred, and about 20 nucleotides appears optimal. Preferably, these sequences will derive from regions which lack heterogeneity. These probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. Among useful probes, for example, are those derived from the newly isolated clones disclosed herein, as well as the various oligomers useful in probing cDNA libraries, set forth below. A complement to any unique portion of the HCV genome will be satisfactory. For use as probes, complete complementarity is desirable, though it may be unnecessary as the length of the fragment is increased.

For use of such probes as diagnostics, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids contained therein. The resulting nucleic acid from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes are then labeled. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The nucleic acids extracted from the sample are then treated with the labeled probe under hybridization conditions of suitable stringencies, and polynucleotide duplexes containing the probe are detected.

The probes can be made completely complementary to the HCV genome. Therefore, usually high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency should only be used if the probes are complementary to regions of the viral genome which lack heterogeneity. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Maniatis, T. (1982).

Generally, it is expected that the HCV genome sequences will be present in serum of infected individuals at relatively low levels, i.e., at approximately 10²-10³ chimp infectious doses (CID) per ml. This level may require that amplification techniques be used in hybridization assays. Such techniques are known in the art. For example, the Enzo Biochemical Corporation "Bio-Bridge" system uses terminal deoxynucleotide transferase to add unmodified 3'-poly-dT-tails to a DNA probe. The poly dT-tailed probe is hybridized to the target nucleotide sequence, and then to a biotin-modified poly-A. PCT application 84/03520 and EPA124221 describe a DNA hybridization assay in which: (1) analyte is annealed to a single-stranded DNA probe that is complementary to an enzyme-labeled oligonucleotide; and (2) the resulting tailed duplex is hybridized to an enzyme-labeled oligonucleotide. EPA 204510 describes a DNA hybridization assay in which analyte DNA is contacted with a probe that has a tail, such as a poly-dT tail, an amplifier strand that has a sequence that hybridizes to the tail of the probe, such as a poly-A sequence, and which is capable of binding a plurality of labeled strands. A particularly desirable technique may first involve amplification of the target HCV sequences in sera approximately 10,000 fold, i.e., to approximately 10⁶ sequences/ml. This may be accomplished, for example, by the polymerase chain reactions (PCR) technique described which is by Saiki et al. (1986), by Mullis, U.S. Patent No. 4,683,195, and by Mullis et al. U.S. Patent No. 4,683,202. The amplified sequence(s) may then be detected using a hybridization assay which is described in EP 317,077, published May 24, 1989. These hybridization assays, which should detect sequences at the level of 10⁶/ml, utilize nucleic acid multimers which bind to single-stranded analyte nucleic acid, and which also bind to a multiplicity of single-stranded labeled oligonucleotides. A suitable solution phase sandwich assay which may be used with labeled polynucleotide probes, and the methods for the preparation of probes is described in EPO 225,807, published June 16, 1987.

The probes can be packaged into diagnostic kits. Diagnostic kits include the probe DNA, which may be

labeled; alternatively, the probe DNA may be unlabeled and the ingredients for labeling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, as well as instructions for conducting the test.

5

Immunoassay and Diagnostic Kits

Both the polypeptides which react immunologically with serum containing HCV antibodies, for example, those detected by the antigenic screening method described infra in the Examples, as well those derived from or encoded within the isolated clones described in the Examples, and composites thereof, and the antibodies raised against the HCV specific epitopes in these polypeptides, are useful in immunoassays to detect presence of HCV antibodies, or the presence of the virus and/or viral antigens, in biological samples. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. For example, the immunoassay may utilize one viral epitope; alternatively, the immunoassay may use a combination of viral epitopes derived from these sources; these epitopes may be derived from the same or from different viral polypeptides, and may be in separate recombinant or natural polypeptides, or together in the same recombinant polypeptides. It may use, for example, a monoclonal antibody directed towards a viral epitope(s), a combination of monoclonal antibodies directed towards epitopes of one viral antigen, monoclonal antibodies directed towards epitopes of different viral antigens, polyclonal antibodies directed towards the same viral antigen, or polyclonal antibodies directed towards different viral antigens. Protocols may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Some of the antigenic regions of the putative polyprotein have been mapped and identified by screening the antigenicity of bacterial expression products of HCV cDNAs which encode portions of the polyprotein. See the Examples. Other antigenic regions of HCV may be detected by expressing the portions of the HCV cDNAs in other expression systems, including yeast systems and cellular systems derived from insects and vertebrates. In addition, studies giving rise to an antigenicity index and hydrophobicity/hydrophilicity profile give rise to information concerning the probability of a region's antigenicity.

The studies on antigenic mapping by expression of HCV cDNAs showed that a number of clones containing these cDNAs expressed polypeptides which were immunologically reactive with serum from individuals with NANBH. No single polypeptide was immunologically reactive with all sera. Five of these polypeptides were very immunogenic in that antibodies to the HCV epitopes in these polypeptides were detected in many different patient sera, although the overlap in detection was not complete. Thus, the results on the immunogenicity of the polypeptides encoded in the various clones suggest that effluent detection systems may include the use of panels of epitopes. The epitopes in the panel may be constructed into one or multiple polypeptides.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the polypeptides of the invention containing HCV epitopes or antibodies directed against HCV epitopes in suitable containers, along with the remaining reagents and materials required for the conduct of the assay, as well as a suitable set of assay instructions.

Further Characterization of the HCV Genome, Virions, and Viral Antigens Using Probes Derived From cDNA to the Viral Genome

The HCV cDNA sequence information in the newly isolated clones described in the Examples may be used to gain further information on the sequence of the HCV genome, and for identification and isolation of the HCV agent, and thus will aid in its characterization including the nature of the genome, the structure of the viral particle, and the nature of the antigens of which it is composed. This information, in turn, can lead to additional polynucleotide probes, polypeptides derived from the HCV genome, and antibodies directed against HCV epitopes which would be useful for the diagnosis and/or treatment of HCV caused NANBH.

The cDNA sequence information in the abovementioned clones is useful for the design of probes for the

isolation of additional cDNA sequences which are derived from as yet undefined regions of the HCV genome(s) from which the cDNAs in clones described herein and in EP 0,316,218 are derived. For example, labeled probes containing a sequence of approximately 8 or more nucleotides, and preferably 20 or more nucleotides, which are derived from regions close to the 5'-termini or 3'-termini of the composite HCV cDNA sequence shown in Fig. 17 may be used to isolate overlapping cDNA sequences from HCV cDNA libraries. Alternatively, characterization of the genomic segments could be from the viral genome(s) isolated from purified HCV particles. Methods for purifying HCV particles and for detecting them during the purification procedure are described herein, infra. Procedures for isolating polynucleotide genomes from viral particles are known in the art, and one procedure which may be used is that described in EP 10 0,218,318. The isolated genomic segments could then be cloned and sequenced. An example of this technique, which utilizes amplification of the sequences to be cloned, is provided infra, and yielded clone 16jh.

Methods for constructing cDNA libraries are known in the art, and are discussed supra and infra; a method for the construction of HCV cDNA libraries in lambda-gt11 is discussed in EPO Pub. No. 318,216. 15 However, cDNA libraries which are useful for screening with nucleic acid probes may also be constructed in other vectors known in the art, for example, lambda-gt10 (Huynh et al. (1985)).

Screening for Anti-Viral Agents for HCV

20 The availability of cell culture and animal model systems for HCV makes it possible to screen for anti-viral agents which inhibit HCV replication, and particularly for those agents which preferentially allow cell growth and multiplication while inhibiting viral replication. These screening methods are known by those of skill in the art. Generally, the anti-viral agents are tested at a variety of concentrations, for their effect on preventing viral replication in cell culture systems which support viral replication, and then for an inhibition of infectivity or of viral pathogenicity (and a low level of toxicity) in an animal model system.

25 The methods and compositions provided herein for detecting HCV antigens and HCV polynucleotides are useful for screening of anti-viral agents in that they provide an alternative, and perhaps more sensitive means, for detecting the agent's effect on viral replication than the cell plaque assay or ID₅₀ assay. For 30 example, the HCV-polynucleotide probes described herein may be used to quantitate the amount of viral nucleic acid produced in a cell culture. This could be accomplished, for example, by hybridization or competition hybridization of the infected cell nucleic acids with a labeled HCV-polynucleotide probe. For example, also, anti-HCV antibodies may be used to identify and quantitate HCV antigen(s) in the cell culture utilizing the immunoassays described herein. In addition, since it may be desirable to quantitate HCV 35 antigens in the infected cell culture by a competition assay, the polypeptides encoded within the HCV cDNAs described herein are useful in these competition assays. Generally, a recombinant HCV polypeptide derived from the HCV cDNA would be labeled, and the inhibition of binding of this labeled polypeptide to an HCV polypeptide due to the antigen produced in the cell culture system would be monitored. Moreover, these techniques are particularly useful in cases where the HCV may be able to replicate in a cell line 40 without causing cell death.

The anti-viral agents which may be tested for efficacy by these methods are known in the art, and include, for example, those which interact with virion components and/or cellular components which are necessary for the binding and/or replication of the virus. Typical anti-viral agents may include, for example, inhibitors of virion polymerase and/or protease(s) necessary for cleavage of the precursor polypeptides. 45 Other anti-viral agents may include those which act with nucleic acids to prevent viral replication, for example, anti-sense polynucleotides, etc.

Antisense polynucleotides molecules are comprised of a complementary nucleotide sequence which allows them to hybridize specifically to designated regions of genomes or RNAs. Antisense polynucleotides 50 may include, for example, molecules that will block protein translation by binding to mRNA, or may be molecules which prevent replication of viral RNA by transcriptase. They may also include molecules which carry agents (non-covalently attached or covalently bound) which cause the viral RNA to be inactive by causing, for example, scissions in the viral RNA. They may also bind to cellular polynucleotides which enhance and/or are required for viral infectivity, replicative ability, or chronicity. Antisense molecules which 55 are to hybridize to HCV derived RNAs may be designed based upon the sequence information of the HCV cDNAs provided herein. The antiviral agents based upon anti-sense polynucleotides for HCV may be designed to bind with high specificity, to be of increased solubility, to be stable, and to have low toxicity. Hence, they may be delivered in specialized systems, for example, liposomes, or by gene therapy. In addition, they may include analogs, attached proteins, substituted or altered bonding between bases, etc.

Other types of drugs may be based upon polynucleotides which "mimic" important control regions of the HCV genome, and which may be therapeutic due to their interactions with key components of the system responsible for viral infectivity or replication.

5

General Methods

The general techniques used in extracting the genome from a virus, preparing and probing a cDNA library, sequencing clones, constructing expression vectors, transforming cells, performing immunological assays such as radioimmunoassays and ELISA assays, for growing cells in culture, and the like are known in the art and laboratory manuals are available describing these techniques. However, as a general guide, the following sets forth some sources currently available for such procedures, and for materials useful in carrying them out.

Both prokaryotic and eukaryotic host cells may be used for expression of desired coding sequences when appropriate control sequences which are compatible with the designated host are used. Among prokaryotic hosts, *E. coli* is most frequently used. Expression control sequences for prokaryotes include promoters, optionally containing operator portions, and ribosome binding sites. Transfer vectors compatible with prokaryotic hosts are commonly derived from, for example, pBR322, a plasmid containing operons conferring ampicillin and tetracycline resistance, and the various pUC vectors, which also contain sequences conferring antibiotic resistance markers. These markers may be used to obtain successful transformants by selection. Commonly used prokaryotic control sequences include the Beta-lactamase (penicillinase) and lactose promoter systems (Chang et al. (1977)), the tryptophan (trp) promoter system (Goeddel et al. (1980)) and the lambda-derived P_L promoter and N gene ribosome binding site (Shimatake et al. (1981)) and the hybrid tac promoter (De Boer et al. (1983)) derived from sequences of the trp and lac UV5 promoters. The foregoing systems are particularly compatible with *E. coli*; if desired, other prokaryotic hosts such as strains of *Bacillus* or *Pseudomonas* may be used, with corresponding control sequences.

Eukaryotic hosts include yeast and mammalian cells in culture systems. *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* are the most commonly used yeast hosts, and are convenient fungal hosts. Yeast compatible vectors carry markers which permit selection of successful transformants by conferring prototrophy to auxotrophic mutants or resistance to heavy metals on wild-type strains. Yeast compatible vectors may employ the 2 micron origin of replication (Broach et al. (1983)), the combination of CEN3 and ARS1 or other means for assuring replication, such as sequences which will result in incorporation of an appropriate fragment into the host cell genome. Control sequences for yeast vectors are known in the art and include promoters for the synthesis of glycolytic enzymes (Hess et al. (1968); Holland et al. (1978)), including the promoter for 3 phosphoglycerate kinase (Hitzeman (1980)). Terminators may also be included, such as those derived from the enolase gene (Holland (1981)). Particularly useful control systems are those which comprise the glyceraldehyde-3 phosphate dehydrogenase (GAPDH) promoter or alcohol dehydrogenase (ADH) regulatable promoter, terminators also derived from GAPDH, and if secretion is desired, leader sequence from yeast alpha factor. In addition, the transcriptional regulatory region and the transcriptional initiation region which are operably linked may be such that they are not naturally associated in the wild-type organism. These systems are described in detail in EPO 120,551, published October 3, 1984; EPO 116,201, published August 22, 1984; and EPO 164,556, published December 18, 1985, all of which are assigned to the herein assignee, and are hereby incorporated herein by reference.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including HeLa cells, Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK) cells, and a number of other cell lines. Suitable promoters for mammalian cells are also known in the art and include viral promoters such as that from Simian Virus 40 (SV40) (Fiers (1978)), Rous sarcoma virus (RSV), adenovirus (ADV), and bovine papilloma virus (BPV). Mammalian cells may also require terminator sequences and poly A addition sequences; enhancer sequences which increase expression may also be included, and sequences which cause amplification of the gene may also be desirable. These sequences are known in the art. Vectors suitable for replication in mammalian cells may include viral replicons, or sequences which insure integration of the appropriate sequences encoding NANBV epitopes into the host genome.

Transformation may be by any known method for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus and transducing a host cell with the virus, and by direct uptake of the polynucleotide. The transformation procedure used depends upon the host to be transformed. For example, transformation of the *E. coli* host cells with lambda-gt11 containing BB-NANBV sequences is discussed in the Example section, infra. Bacterial transformation by direct uptake generally employs

treatment with calcium or rubidium chloride (Cohen (1972); Maniatis (1982)). Yeast transformation by direct uptake may be carried out using the method of Hinnen et al. (1978). Mammalian transformations by direct uptake may be conducted using the calcium phosphate precipitation method of Graham and Van der Eb (1978), or the various known modifications thereof.

5 Vector construction employs techniques which are known in the art. Site-specific DNA cleavage is performed by treating with suitable restriction enzymes under conditions which generally are specified by the manufacturer of these commercially available enzymes. In general, about 1 microgram of plasmid or DNA sequence is cleaved by 1 unit of enzyme in about 20 microliters buffer solution by incubation of 1-2 hr at 37°C. After incubation with the restriction enzyme, protein is removed by phenol/chloroform extraction 10 and the DNA recovered by precipitation with ethanol. The cleaved fragments may be separated using polyacrylamide or agarose gel electrophoresis techniques, according to the general procedures found in Methods in Enzymology (1980) 65:499-560.

Sticky ended cleavage fragments may be blunt ended using *E. coli* DNA polymerase I (Klenow) in the presence of the appropriate deoxynucleotide triphosphates (dNTPs) present in the mixture. Treatment with 15 S1 nuclease may also be used, resulting in the hydrolysis of any single stranded DNA portions.

Ligations are carried out using standard buffer and temperature conditions using T4 DNA ligase and ATP; sticky end ligations require less ATP and less ligase than blunt end ligations. When vector fragments are used as part of a ligation mixture, the vector fragment is often treated with bacterial alkaline phosphatase (BAP) or calf intestinal alkaline phosphatase to remove the 5'-phosphate and thus prevent 20 religation of the vector; alternatively, restriction enzyme digestion of unwanted fragments can be used to prevent ligation.

Ligation mixtures are transformed into suitable cloning hosts, such as *E. coli*, and successful transformants selected by, for example, antibiotic resistance, and screened for the correct construction.

Synthetic oligonucleotides may be prepared using an automated oligonucleotide synthesizer as described by Warner (1984). If desired the synthetic strands may be labeled with ^{32}P by treatment with 25 polynucleotide kinase in the presence of ^{32}P -ATP, using standard conditions for the reaction.

DNA sequences, including those isolated from cDNA libraries, may be modified by known techniques, including, for example site directed mutagenesis, as described by Zoller (1982). Briefly, the DNA to be modified is packaged into phage as a single stranded sequence, and converted to a double stranded DNA 30 with DNA polymerase using, as a primer, a synthetic oligonucleotide complementary to the portion of the DNA to be modified, and having the desired modification included in its own sequence. The resulting double stranded DNA is transformed into a phage supporting host bacterium. Cultures of the transformed bacteria, which contain replications of each strand of the phage, are plated in agar to obtain plaques. Theoretically, 50% of the new plaques contain phage having the mutated sequence, and the remaining 50% 35 have the original sequence. Replicates of the plaques are hybridized to labeled synthetic probe at temperatures and conditions which permit hybridization with the correct strand, but not with the unmodified sequence. The sequences which have been identified by hybridization are recovered and cloned.

DNA libraries may be probed using the procedure of Grunstein and Hogness (1975). Briefly, in this 40 procedure, the DNA to be probed is immobilized on nitrocellulose filters, denatured, and prehybridized with a buffer containing 0-50% formamide, 0.75 M NaCl, 75 mM Na citrate, 0.02% (wt/v) each of bovine serum albumin, polyvinyl pyrrolidone, and Ficoll, 50 mM Na Phosphate (pH 6.5), 0.1% SDS, and 100 micrograms/ml carrier denatured DNA. The percentage of formamide in the buffer, as well as the time and 45 temperature conditions of the prehybridization and subsequent hybridization steps depends on the stringency required. Oligomeric probes which require lower stringency conditions are generally used with low percentages of formamide, lower temperatures, and longer hybridization times. Probes containing more than 30 or 40 nucleotides such as those derived from cDNA or genomic sequences generally employ higher temperatures, e.g., about 40-42°C, and a high percentage, e.g., 50%, formamide. Following prehybridization, 5 ^{32}P -labeled oligonucleotide probe is added to the buffer, and the filters are incubated in this mixture under hybridization conditions. After washing, the treated filters are subjected to autoradiography to show 50 the location of the hybridized probe; DNA in corresponding locations on the original agar plates is used as the source of the desired DNA.

For routine vector constructions, ligation mixtures are transformed into *E. coli* strain HB101 or other 55 suitable host, and successful transformants selected by antibiotic resistance or other markers. Plasmids from the transformants are then prepared according to the method of Clewell et al. (1969), usually following chloramphenicol amplification (Clewell (1972)). The DNA is isolated and analyzed, usually by restriction enzyme analysis and/or sequencing. Sequencing may be by the dideoxy method of Sanger et al. (1977) as further described by Messing et al. (1981), or by the method of Maxam et al. (1980). Problems with band compression, which are sometimes observed in GC rich regions, were overcome by use of T-

deazoguanosine according to Barr et al. (1986).

The enzyme-linked immunosorbent assay (ELISA) can be used to measure either antigen or antibody concentrations. This method depends upon conjugation of an enzyme to either an antigen or an antibody, and uses the bound enzyme activity as a quantitative label. To measure antibody, the known antigen is fixed to a solid phase (e.g., a microplate or plastic cup), incubated with test serum dilutions, washed, incubated with anti-immunoglobulin labeled with an enzyme, and washed again. Enzymes suitable for labeling are known in the art, and include, for example, horseradish peroxidase. Enzyme activity bound to the solid phase is measured by adding the specific substrate, and determining product formation or substrate utilization colorimetrically. The enzyme activity bound is a direct function of the amount of antibody bound.

To measure antigen, a known specific antibody is fixed to the solid phase, the test material containing antigen is added, after an incubation the solid phase is washed, and a second enzyme-labeled antibody is added. After washing, substrate is added, and enzyme activity is estimated colorimetrically, and related to antigen concentration.

15

Examples

20 Described below are examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention. In light of the present disclosure, numerous embodiments within the scope of the claims will be apparent to those of ordinary skill in the art.

25 Isolation and Sequence of Overlapping HCV cDNA Clones 13i, 26j, CA59a, CA84a, CA156e and CA167b

The clones 13i, 26j, CA59a, CA84a, CA156e and CA167b were isolated from the lambda-gt11 library which contains HCV cDNA (ATCC No. 40394), the preparation of which is described in EPO Pub. No. 30 318,216 (published 31 May 1989), and WO 89/04669 (published 1 June 1989). Screening of the library was with the probes described infra., using the method described in Huynh (1985). The frequencies with which positive clones appeared with the respective probes was about 1 in 50,000.

The isolation of clone 13i was accomplished using a synthetic probe derived from the sequence of clone 12f. The sequence of the probe was:

35 5' GAA CGT TGC GAT CTG GAA GAC AGG GAC AGG 3'.

The isolation of clone 26j was accomplished using a probe derived from the 5'-region of clone K9-1. The sequence of the probe was:

5' TAT CAG TTA TGC CAA CGG CCC CGA 3'.

The isolation procedures for clone 12f and for clone K9-1 (also called K9-1) are described in EPO Pub. 40 No. 318,216, and their sequences are shown in Figs. 1 and 2, respectively. The HCV cDNA sequences of clones 13i and 26j, are shown in Figs. 4 and 5, respectively. Also shown are the amino acids encoded therein, as well as the overlap of clone 13i with clone 12f, and the overlap of clone 26j with clone 13i. The sequences for these clones confirmed the sequence of clone K9-1. Clone K9-1 had been isolated from a different HCV cDNA library (See EP 0,218,316).

45 Clone CA59a was isolated utilizing a probe based upon the sequence of the 5'-region of clone 26j. The sequence of this probe was:

5' CTG GTT AGC AGG GCT TTT CTA TCA CCA CAA 3'.

A probe derived from the sequence of clone CA59a was used to isolate clone CA84a. The sequence of the probe used for this isolation was:

50 5' AAG GTC CTG GTA GTG CTG CTG CTA TTT GCC 3'.

Clone CA156e was isolated using a probe derived from the sequence of clone CA84a. The sequence of the probe was:

5' ACT GGA CGA CGC AAG GTT GCA ATT GCT CTA 3'.

Clone CA167b was isolated using a probe derived from the sequence of clone CA 156e. The sequence of the probe was:

55 5' TTC GAC GTC ACA TCG ATC TGC TTG TCG GGA 3'.

The nucleotide sequences of the HCV cDNAs in clones CA59a, CA84a, CA156e, and CA167b, are shown Figs. 6, 7, 8, and 9, respectively. The amino acids encoded therein, as well as the overlap with the

sequences of relevant clones, are also shown in the Figs.

Creation of "pi" HCV cDNA Library

5

A library of HCV cDNA, the "pi" library, was constructed from the same batch of infectious chimpanzee plasma used to construct the lambda-gt11 HCV cDNA library (ATCC No. 40394) described in EPO Pub. No. 318,216, and utilizing essentially the same techniques. However, construction of the pi library utilized a 10 primer-extension method, in which the primer for reverse transcriptase was based on the sequence of clone CA59A. The sequence of the primer was:
5' GGT GAC GTG GGT TTC 3'.

15

Isolation and Sequence of Clone pi14a

Screening of the "pi" HCV cDNA library described supra., with the probe used to isolate clone CA167b (See supra.) yielded clone pi14a. The clone contains about 800 base pairs of cDNA which overlaps clones 20 CA167b, CA156e, CA84a and CA59a, which were isolated from the lambda gt-11 HCV cDNA library (ATCC No. 40394). In addition, pi14a also contains about 250 base pairs of DNA which are upstream of the HCV cDNA in clone CA167b.

25

Isolation and Sequence of Clones CA216a, CA290a and ag30a

Based on the sequence of clone CA167b a synthetic probe was made having the following sequence:
5' GGC TTT ACC ACG TCA CCA ATG ATT GCC CTA 3'
30 The above probe was used to screen the lambda-gt11 library (ATCC No. 40394), which yielded clone CA216a, whose HCV sequences are shown in Fig. 10.

Another probe was made based on the sequence of clone CA216a having the following sequence:
5' TTT GGG TAA GGT CAT CGA TAC CCT TAC GTG 3'
Screening the lambda-gt11 library (ATCC No. 40394) with this probe yielded clone CA290a, the HCV 35 sequences therein being shown in Fig. 11.

In a parallel approach, a primer-extension cDNA library was made using nucleic acid extracted from the same infectious plasma used in the original lambda-gt11 cDNA library described above. The primer used was based on the sequence of clones CA216a and CA290a:

5' GAA GCC GCA CGT AAG 3'
40 The cDNA library was made using methods similar to those described previously for libraries used in the isolation of clones pi14a and k9-1. The probe used to screen this library was based on the sequence of clone CA290a:
5' CCG GCG TAG GTC GCG CAA TTT GGG TAA 3'
Clone ag30a was isolated from the new library with the above probe, and contained about 670 basepairs of 45 HCV sequence. See Fig. 12. Part of this sequence overlaps the HCV sequence of clones CA216a and CA290a. About 300 base-pairs of the ag30a sequence, however, is upstream of the sequence from clone CA290a. The non-overlapping sequence shows a start codon (*) and stop codons that may indicate the start of the HCV ORF. Also indicated in Fig. 12 are putative small encoded peptides (#) which may play a role in regulating translation, as well as the putative first amino acid of the putative polypeptide (/), and downstream 50 amino acids encoded therein.

Isolation and Sequence of Clone CA205a

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Clone CA205a was isolated from the original lambda gt-11 library (ATCC No. 40394), using a synthetic probe derived from the HCV sequence in clone CA290a (Fig. 11). The sequence of the probe was:
5' TCA GAT CGT TGG TGG AGT TTA CTT GTT GCC 3'.

The sequence of the HCV cDNA in CA205a, shown in Fig. 13, overlaps with the cDNA sequences in both clones ag30a and CA290a. The overlap of the sequence with that of CA290a is shown by the dotted line above the sequence (the figure also shows the putative amino acids encoded in this fragment).

As observed from the HCV cDNA sequences in clones CA205a and ag30a, the putative HCV polyprotein appears to begin at the ATG start codon; the HCV sequences in both clones contain an in-frame, contiguous double stop codon (TGATAG) forty two nucleotides upstream from this ATG. The HCV ORF appears to begin after these stop codons, and to extend for at least 8907 nucleotides (See the composite HCV cDNA shown in Fig. 17).

10

Isolation and Sequence of Clone 18g

Based on the sequence of clone ag30a (See Fig. 12) and of an overlapping clone from the original lambda gt-11 library (ATCC No. 40394), CA230a, a synthetic probe was made having the following sequence:

5' CCA TAG TGG TCT GCG GAA CCG GTG AGT ACA 3'.

Screening of the original lambda-gt11 HCV cDNA library with the probe yielded clone 18g, the HCV cDNA sequence of which is shown in Fig. 14. Also shown in the figure are the overlap with clone ag30a, and putative polypeptides encoded within the HCV cDNA.

The cDNA in clone 18g (C18g or 18g) overlaps that in clones ag30a and CA205a, described supra. The sequence of C18g also contains the double stop codon region observed in clone ag30a. The polynucleotide region upstream of these stop codons presumably represents part of the 5'-region of the HCV genome, which may contain short ORFs, and which can be confirmed by direct sequencing of the purified HCV genome. These putative small encoded peptides may play a regulatory role in translation. The region of the HCV genome upstream of that represented by C18g can be isolated for sequence analysis using essentially the technique described in EPO Pub. No. 318,216 for isolating cDNA sequences upstream of the HCV cDNA sequence in clone 12f. Essentially, small synthetic oligonucleotide primers of reverse transcriptase, which are based upon the sequence of C18g, are synthesized and used to bind to the corresponding sequence in HCV genomic RNA. The primer sequences are proximal to the known 5'-terminal of C18g, but sufficiently downstream to allow the design of probe sequences upstream of the primer sequences. Known standard methods of priming and cloning are used. The resulting cDNA libraries are screened with sequences upstream of the priming sites (as deduced from the elucidated sequence of C18g). The HCV genomic RNA is obtained from either plasma or liver samples from individuals with NANBH. Since HCV appears to be a Flavi-like virus, the 5'-terminus of the genome may be modified with a "cap" structure. It is known that Flavivirus genomes contain 5'-terminal "cap" structures. (Yellow Fever virus, Rice et al. (1988); Dengue virus, Hahn et al (1988); Japanese Encephalitis Virus (1987)).

40

Isolation and Sequence of Clones from the beta-HCV cDNA library

Clones containing cDNA representative of the 3'-terminal region of the HCV genome were isolated from a cDNA library constructed from the original infectious chimpanzee plasma pool which was used for the creation of the HCV cDNA lambda-gt11 library (ATCC No. 40394), described in EPO Pub. No. 318,216. In order to create the DNA library, RNA extracted from the plasma was "tailed" with poly rA using poly (rA) polymerase, and cDNA was synthesized using oligo(dT)₁₂₋₁₈ as a primer for reverse transcriptase. The resulting RNA:cDNA hybrid was digested with RNase H, and converted to double stranded HCV cDNA. The resulting HCV cDNA was cloned into lambda-gt10, using essentially the technique described in Huynh (1985), yielding the beta (or b) HCV cDNA library. The procedures used were as follows.

An aliquot (12ml) of the plasma was treated with proteinase K, and extracted with an equal volume of phenol saturated with 0.05M Tris-Cl, pH 7.5, 0.05% (v/v) beta-mercaptoethanol, 0.1% (w/v) hydroxyquinolone, 1 mM EDTA. The resulting aqueous phase was re-extracted with the phenol mixture, followed by 3 extractions with a 1:1 mixture containing phenol and chloroform:isoamyl alcohol (24:1), followed by 2 extractions with a mixture of chloroform and isoamyl alcohol (1:1). Subsequent to adjustment of the aqueous phase to 200 mM with respect to NaCl, nucleic acids in the aqueous phase were precipitated overnight at -20°C, with 2.5 volumes of cold absolute ethanol. The precipitates were collected by centrifugation at 10,000 RPM for 40 min., washed with 70% ethanol containing 20 mM NaCl, and with 100% cold ethanol,

dried for 5 min. in a dessicator, and dissolved in water.

The isolated nucleic acids from the infectious chimpanzee plasma pool were tailed with poly rA utilizing poly-A polymerase in the presence of human placenta ribonuclease inhibitor (HPRI) (purchased from Amersham Corp.), utilizing MS2 RNA as carrier. Isolated nucleic acids equivalent to that in 2 ml of plasma were incubated in a solution containing TMN (50 mM Tris HCl, pH 7.9, 10 mM MgCl₂, 250 mM NaCl, 2.5 mM MnCl₂, 2 mM dithiothreitol (DTT)), 40 micromolar alpha-[³²P] ATP, 20 units HPRI (Amersham Corp.), and about 9 to 10 units of RNase free poly-A polymerase (BRL). Incubation was for 10 min. at 37 °C, and the reactions were stopped with EDTA (final concentration about 250 mM). The solution was extracted with an equal volume of phenol-chloroform, and with an equal volume of chloroform, and nucleic acids were precipitated overnight at -20 °C with 2.5 volumes of ethanol in the presence of 200 mM NaCl.

Isolation of Clone b5a

The beta HCV cDNA library was screened by hybridization using a synthetic probe, which had a sequence based upon the HCV cDNA sequence in clone 15e. The isolation of clone 15e is described in EPO Pub. No. 318,216, and its sequence is shown in Fig. 3. The sequence of the synthetic probe was:
 5' ATT GCG AGA TCT ACG GGG CCT GCT ACT CCA 3'.
 Screening of the library yielded clone beta-5a (b5a), which contains an HCV cDNA region of approximately 1000 base pairs. The 5'-region of this cDNA overlaps clones 35f, 19g, 26g, and 15e (these clones are described supra). The region between the 3'-terminal poly-A sequence and the 3'-sequence which overlaps clone 15e, contains approximately 200 base pairs. This clone allows the identification of a region of the 3'-terminal sequence the HCV genome.
 The sequence of b5a is contained within the sequence of the HCV cDNA in clone 16jh (described infra). Moreover, the sequence is also present in CC34a, isolated from the original lambda-gt11 library (ATCC No. 40394). (The original lambda-gt11 library is referred to herein as the "C" library).

Isolation and Sequence of Clones Generated by PCR Amplification of the 3'-Region of the HCV Genome

Multiple cDNA clones have been generated which contain nucleotide sequences derived from the 3'-region of the HCV genome. This was accomplished by amplifying a targeted region of the genome by a polymerase chain reaction technique described in Saiki et al. (1986), and in Saiki et al. (1988), which was modified as described below. The HCV RNA which was amplified was obtained from the original infectious chimpanzee plasma pool which was used for the creation of the HCV cDNA lambda-gt11 library (ATCC No. 40394) described in EPO Pub. No. 318,216. Isolation of the HCV RNA was as described supra. The isolated RNA was tailed at the 3'-end with ATP by *E. coli* poly-A polymerase as described in Sippel (1973), except that the nucleic acids isolated from chimp serum were substituted for the nucleic acid substrate. The tailed RNA was then reverse transcribed into cDNA by reverse transcriptase, using an oligo dT-primer adapter, essentially as described by Han (1987), except that the components and sequence of the primer-adapter were:

Stuffer	NotI	SP6 Promoter	Primer
AATTC	GCGGCCGC	CATACGATTAGGTGACACTATAGAA	T ₁₅

The resultant cDNA was subjected to amplification by PCR using two primers:

Primer	Sequence
JH32 (30mer)	ATAGCGGCCGCCCTCGATTGCGAGATCTAC
JH11 (20mer)	AATTCGGCGGCCCATACGA

The JH32 primer contained 20 nucleotide sequences hybridizable to the 5'-end of the target region in the cDNA, with an estimated T_m of 66°C. The JH11 was derived from a portion of the oligo dT-primer adapter; thus, it is specific to the 3'-end of the cDNA with a T_m of 64°C. Both primers were designed to have a recognition site for the restriction enzyme, NotI, at the 5'-end, for use in subsequent cloning of the amplified HCV cDNA.

The PCR reaction was carried out by suspending the cDNA and the primers in 100 microliters of reaction mixture containing the four deoxynucleoside triphosphates, buffer salts and metal ions, and a thermostable DNA polymerase isolated from *Thermus aquaticus* (Taq polymerase), which are in a Perkin Elmer Cetus PCR kit (N801-0043 or N801-0055). The PCR reaction was performed for 35 cycles in a Perkin 10 Elmer Cetus DNA thermal cycler. Each cycle consisted of a 1.5 min denaturation step at 94°C, an annealing step at 60°C for 2 min, and a primer extension step at 72°C for 3 min. The PCR products were subjected to Southern blot analysis using a 30 nucleotide probe, JH34, the sequence of which was based upon that of the 3'-terminal region of clone 15e. The sequence of JH34 is:
5' CTT GAT CTA CCT CCA ATC ATT CAA AGA CTC 3.

15 The PCR products detected by the HCV cDNA probe ranged in size from about 50 to about 400 base pairs.

In order to clone the amplified HCV cDNA, the PCR products were cleaved with NotI and size selected by polyacrylamide gel electrophoresis. DNA larger than 300 base pairs was cloned into the NotI site of pUC18S. The vector pUC18S is constructed by including a NotI polylinker cloned between the EcoRI and Sall sites of pUC18. The clones were screened for HCV cDNA using the JH34 probe. A number of positive 20 clones were obtained and sequenced. The nucleotide sequence of the HCV cDNA insert in one of these clones, 16jh, and the amino acids encoded therein, are shown in Fig. 15. A nucleotide heterogeneity, detected in the sequence of the HCV cDNA in clone 16jh as compared to another clone of this region, is indicated in the figure.

25

Compiled HCV cDNA Sequences

An HCV cDNA sequence has been compiled from a series of overlapping clones derived from the 30 various HCV cDNA libraries described supra.. In this sequence, the compiled HCV cDNA sequence obtained from clones b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, and CA59a is upstream of the compiled HCV cDNA sequence published in EPO Pub. No. 318,216, which is shown in Fig. 16. The compiled HCV cDNA sequence obtained from clones b5a and 16jh downstream of the compiled HCV cDNA sequence published in EPO Pub. No. 318,216.

35 Fig. 17 shows the compiled HCV cDNA sequence derived from the above-described clones and the compiled HCV cDNA sequence published in EPO Pub. No. 318,216. The clones from which the sequence was derived are b114a, 18g, ag30a, CA205a, CA290a, CA216a, pi14a, CA167b, CA156e, CA84a, CA59a, K9-1 (also called k9-1), 26j, 13i, 12f, 14i, 11b, 7f, 7e, 8h, 33c, 40b, 37b, 35, 36, 81, 32, 33b, 25c, 14c, 8f, 33f, 40 33g, 39c, 35f, 19g, 26g, 15e, b5a, and 16jh. In the figure the three dashes above the sequence indicate the position of the putative initiator methionine codon.

Clone b114a was obtained using the cloning procedure described for clone b5a, supra., except that the probe was the synthetic probe used to detect clone 18g, supra. Clone b114a overlaps with clones 18g, ag30a, and CA205a, except that clone b114a contains an extra two nucleotides upstream of the sequence in clone 18g (i.e., 5'-CA). These extra two nucleotides have been included in the HCV genomic sequence 45 shown in Fig. 17.

It should be noted that although several of the clones described supra. have been obtained from libraries other than the original HCV cDNA lambda-gt11 C library (ATCC No. 40394), these clones contain HCV cDNA sequences which overlap HCV cDNA sequences in the original library. Thus, essentially all of the HCV sequence is derivable from the original lambda-gt11 C library (ATCC No. 40394) which was used 50 to isolate the first HCV cDNA clone (5-1-1). The isolation of clone 5-1-1 is described in EPO Pub. No. 318,216.

Purification of Fusion polypeptide C100-3 (Alternate method)

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The fusion polypeptide, C100-3 (also called HCV c100-3 and alternatively, c100-3), is comprised of superoxide dismutase (SOD) at the N-terminus and in-frame C100 HCV polypeptide at the C-terminus. A

method for preparing the polypeptide by expression in yeast, and differential extraction of the insoluble fraction of the extracted host yeast cells, is described in EPO Pub. No. 318,216. An alternative method for the preparation of this fusion polypeptide is described below. In this method the antigen is precipitated from the crude cell lysate with acetone; the acetone precipitated antigen is then subjected to ion-exchange chromatography, and further purified by gel filtration.

The fusion polypeptide, C100-3 (HCV c100-3), is expressed in yeast strain JSC 308 (ATCC No. 20879) transformed with pAB24C100-3 (ATCC No. 67976); the transformed yeast are grown under conditions which allow expression (i.e., by growth in YEP containing 1% glucose). (See EPO Pub. No. 318,216). A cell lysate is prepared by suspending the cells in Buffer A (20 mM Tris HCl, pH 8.0, 1 mM EDTA, 1 mM PMSF. The cells are broken by grinding with glass beads in a Dynomill type homogenizer or its equivalent. The extent of cell breakage is monitored by counting cells under a microscope with phase optics. Broken cells appear dark, while viable cells are light-colored. The percentage of broken cells is determined.

When the percentage of broken cells is approximately 90% or greater, the broken cell debris is separated from the glass beads by centrifugation, and the glass beads are washed with Buffer A. After combining the washes and homogenate, the insoluble material in the lysate is obtained by centrifugation. The material in the pellet is washed to remove soluble proteins by suspension in Buffer B (50 mM glycine, pH 12.0, 1 mM DTT, 500 mM NaCl), followed by Buffer C (50 mM glycine, pH 10.0, 1 mM DTT). The insoluble material is recovered by centrifugation, and solubilized by suspension in Buffer C containing SDS. The extract solution may be heated in the presence of beta-mercaptoethanol and concentrated by ultrafiltration. The HCV c100-3 in the extract is precipitated with cold acetone. If desired, the precipitate may be stored at temperatures at about or below -15 °C.

Prior to ion exchange chromatography, the acetone precipitated material is recovered by centrifugation, and may be dried under nitrogen. The precipitate is suspended in Buffer D (50 mM glycine, pH 10.0, 1 mM DTT, 7 M urea), and centrifuged to pellet insoluble material. The supernatant material is applied to an anion exchange column previously equilibrated with Buffer D. Fractions are collected and analyzed by ultraviolet absorbance or gel electrophoresis on SDS polyacrylamide gels. Those fractions containing the HCV c100-3 polypeptide are pooled.

In order to purify the HCV c100-3 polypeptide by gel filtration, the pooled fractions from the ion-exchange column are heated in the presence of beta-mercaptoethanol and SDS, and the eluate is concentrated by ultrafiltration. The concentrate is applied to a gel filtration column previously equilibrated with Buffer E (20 mM Tris HCl, pH 7.0, 1 mM DTT, 0.1% SDS). The presence of HCV c100-3 in the eluted fractions, as well as the presence of impurities, are determined by gel electrophoresis on polyacrylamide gels in the presence of SDS and visualization of the polypeptides. Those fractions containing purified HCV c100-3 are pooled. Fractions high in HCV c100-3 may be further purified by repeating the gel filtration process. If the removal of particulate material is desired, the HCV c100-3 containing material may be filtered through a 0.22 micron filter.

Expression and Antigenicity of Polypeptides Encoded in HCV cDNA

40

Polypeptides Expressed in E. coli

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The polypeptides encoded in a number of HCV cDNAs which span the HCV genomic ORF were expressed in E. coli, and tested for their antigenicity using serum obtained from a variety of individuals with NANBH. The expression vectors containing the cloned HCV cDNAs were constructed from pSODcf1 (Steimer et al. (1986)). In order to be certain that a correct reading frame would be achieved, three separate expression vectors, pcf1AB, pcf1CD, and pcf1Ef were created by ligating either of three linkers, AB, CD, and EF to a BamHI-EcoRI fragment derived by digesting to completion the vector pSODcf1 with EcoRI and BamHI, followed by treatment with alkaline phosphatase. The linkers were created from six oligomers, A, B, C, D, E, and F. Each oligomer was phosphorylated by treatment with kinase in the presence of ATP prior to annealing to its complementary oligomer. The sequences of the synthetic linkers were the following.

55

Name	DNA Sequence (5' to 3')
5	A GATC CTG AAT TCC TGA TAA B GAC TTA AGG ACT ATT TTA A
10	C GATC CGA ATT CTG TGA TAA D GCT TAA GAC ACT ATT TTA A
15	E GATC CTG GAA TTC TGA TAA F GAC CTT AAG ACT ATT TTA A

Each of the three linkers destroys the original EcoRI site, and creates a new EcoRI site within the linker, but within a different reading frame. Hence, the HCV cDNA EcoRI fragments isolated from the clones when inserted into the expression vector, were in three different reading frames.

20 The HCV cDNA fragments in the designated lambda-gt11 clones were excised by digestion with EcoRI; each fragment was inserted into pcf1AB, pcf1CD, and pcf1EF. These expression constructs were then transformed into D1210 E. coli cells, the transformants were cloned, and recombinant bacteria from each clone were induced to express the fusion polypeptides by growing the bacteria in the presence of IPTG.

25 Expression products of the indicated HCV cDNAs were tested for antigenicity by direct immunological screening of the colonies, using a modification of the method described in Helfman et al. (1983). Briefly, as shown in Fig. 18, the bacteria were plated onto nitrocellulose filters overlaid on ampicillin plates to give approximately 1,000 colonies per filter. Colonies were replica plated onto nitrocellulose filters, and the replicas were regrown overnight in the presence of 2 mM IPTG and ampicillin. The bacterial colonies were lysed by suspending the nitrocellulose filters for about 15 to 20 min in an atmosphere saturated with CHCl₃ vapor. Each filter then was placed in an individual 100 mm Petri dish containing 10 ml of 50 mM Tris HCl, pH 7.5, 150 mM NaCl, 5 mM MgCl₂, 3% (w/v) BSA, 40 micrograms/ml lysozyme, and 0.1 microgram/ml DNase. The plates were agitated gently for at least 8 hours at room temperature. The filters were rinsed in TBST (50 mM Tris HCl, pH 8.0, 150 mM NaCl, 0.005% Tween 20). After incubation, the cell residues were rinsed and incubated in TBS (TBST without Tween) containing 10% sheep serum; incubation was for 1 hour. The filters were then incubated with pretreated sera in TBS from individuals with NANBH, which included: 3 chimpanzees; 8 patients with chronic NANBH whose sera were positive with respect to antibodies to HCV C100-3 polypeptide (described in EPO Pub. No. 318,216, and supra.) (also called C100); 8 patients with chronic NANBH whose sera were negative for anti-C100 antibodies; a convalescent patient whose serum was negative for anti-C100 antibodies; and 6 patients with community acquired NANBH, including one whose sera was strongly positive with respect to anti-C100 antibodies, and one whose sera was marginally positive with respect to anti-C100 antibodies. The sera, diluted in TBS, was pretreated by preabsorption with hSOD. Incubation of the filters with the sera was for at least two hours. After incubation, the filters were washed two times for 30 min with TBST. Labeling of expressed proteins to which antibodies in the sera bound was accomplished by incubation for 2 hours with ¹²⁵I-labeled sheep anti-human antibody. After washing, the filters were washed twice for 30 min with TBST, dried, and autoradiographed.

50 A number of clones (see infra.) expressed polypeptides containing HCV epitopes which were immunologically reactive with serum from individuals with NANBH. Five of these polypeptides were very immunogenic in that antibodies to HCV epitopes in these polypeptides were detected in many different patient sera. The clones encoding these polypeptides, and the location of the polypeptide in the putative HCV polyprotein (wherein the amino acid numbers begin with the putative initiator codon) are the following: clone 5-1-1, amino acids 1694-1735; clone C100, amino acids 1569-1931; clone 33c, amino acids 1192-1457; clone CA279a, amino acids 1-84; and clone CA290a amino acids 9-177. The location of the immunogenic polypeptides within the putative HCV polyprotein are shown immediately below.

Clones encoding polypeptides of proven reactivity with sera from NANBH patients.	
Clone	Location within the HCV polyprotein
	(amino acid no. beginning with putative initiator methionine)
CA279a	1-84
CA74a	437-582
13i	511-690
CA290a	9-177
33c	1192-1457
40b	1266-1428
5-1-1	1694-1735
81	1689-1805
33b	1916-2021
25c	1949-2124
14c	2054-2223
8f	2200-3325
33f	2287-2385
33g	2348-2464
39c	2371-2502
15e	2796-2886
C100	1569-1931

The results on the immunogenicity of the polypeptides encoded in the various clones examined suggest efficient detection and immunization systems may include panels of HCV polypeptides/epitopes.

Expression of HCV Epitopes in Yeast

Three different yeast expression vectors which allow the insertion of HCV cDNA into three different reading frames are constructed. The construction of one of the vectors, pAB24C100-3 is described in EPO Pub. No. 318,216. In the studies below, the HCV cDNA from the clones listed in supra, in the antigenicity mapping study using the *E. coli* expressed products are substituted for the C100 HCV cDNA. The construction of the other vectors replaces the adaptor described in the above *E. coli* studies with one of the following adaptors:

Adaptor 1

ATT TTG AAT TCC TAA TGA G
AC TTA AGG ATT ACT CAG CT

Adaptor 2

AAT TTG GAA TTC TAA TGA G
AC CTT AAG ATT ACT CAG CT.

The inserted HCV cDNA is expressed in yeast transformed with the vectors, using the expression

conditions described supra. for the expression of the fusion polypeptide, C100-3. The resulting polypeptides are screened using the sera from individuals with NANBH, described supra. for the screening of immunogenic polypeptides encoded in HCV cDNAs expressed in E. coli.

5

Comparison of the Hydrophobic Profiles of HCV Polyproteins with West Nile Virus Polyprotein and with Dengue Virus NS1

10 The hydrophobicity profile of an HCV polyprotein segment was compared with that of a typical Flavivirus, West Nile virus. The polypeptide sequence of the West Nile virus polyprotein was deduced from the known polynucleotide sequences encoding the non-structural proteins of that virus. The HCV polyprotein sequence was deduced from the sequence of overlapping cDNA clones. The profiles were determined using an antigen program which uses a window of 7 amino acid width (the amino acid in question, and 3 residues on each side) to report the average hydrophobicity about a given amino acid residue. The parameters giving the reactive hydrophobicity for each amino acid residue are from Kyte and Doolittle (1982). Fig. 19 shows the hydrophobic profiles of the two polyproteins; the areas corresponding to the non-structural proteins of West Nile virus, ns1 through ns5, are indicated in the figure. As seen in the figure, there is a general similarity in the profiles of the HCV polyprotein and the West Nile virus polyprotein.

15 20 The sequence of the amino acids encoded in the 5'-region of HCV cDNA shown in Fig. 16 has been compared with the corresponding region of one of the strains of Dengue virus, described supra., with respect to the profile of regions of hydrophobicity and hydrophilicity (data not shown). This comparison indicated that the polypeptides from HCV and Dengue encoded in this region, which corresponds to the region encoding NS1 (or a portion thereof), have a similar hydrophobic/hydrophilic profile.

25 The similarity in hydrophobicity profiles, in combination with the previously identified homologies in the amino acid sequences of HCV and Dengue Flavivirus in EP 0,218,316 suggests that HCV is related to these members of the Flavivirus family.

30

Characterization of the Putative Polypeptides Encoded Within the HCV ORF

35 The sequence of the HCV cDNA sense strand, shown in Fig. 17, was deduced from the overlapping HCV cDNAs in the various clones described in EPO Pub. No. 318,216 and those described supra. It may be deduced from the sequence that the HCV genome contains primarily one long continuous ORF, which encodes a polyprotein. In the sequence, nucleotide number 1 corresponds to the first nucleotide of the initiator MET codon; minus numbers indicate that the nucleotides are that distance away in the 5'-direction (upstream), while positive numbers indicate that the nucleotides are that distance away in the 3'-direction (downstream). The composite sequence shows the "sense" strand of the HCV cDNA.

40 The amino acid sequence of the putative HCV polyprotein deduced from the HCV cDNA sense strand sequence is also shown in Fig. 17, where position 1 begins with the putative initiator methionine.

Possible protein domains of the encoded HCV polyprotein, as well as the approximate boundaries, are the following (the polypeptides identified within the parentheses are those which are encoded in the Flavivirus domain):

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50

55

	Putative Domain	Approximate Boundary
		(amino acid nos.)
5	"C" (nucleocapsid protein)	1-120
	"E" (Virion envelope protein(s) and possibly matrix (M) proteins	120-400
	"NS1" (complement fixation antigen?)	400-660
	"NS2" (unknown function)	660-1050
	"NS3" (protease?)	1050-1640
	"NS4" (unknown function)	1640-2000
	"NS5" (polymerase)	2000-? end

15 It should be noted, however, that hydrophobicity profiles (described infra), indicate that HCV diverges from the Flavivirus model, particularly with respect to the region upstream of NS2. Moreover, the boundaries indicated are not intended to show firm demarcations between the putative polypeptides.

20 The Hydrophilic and Antigenic Profile of the Polypeptide

25 Profiles of the hydrophilicity/hydrophobicity and the antigenic index of the putative polyprotein encoded in the HCV cDNA sequence shown in Fig. 16 were determined by computer analysis. The program for hydrophilicity/hydrophobicity was as described supra. The antigenic index results from a computer program which relies on the following criteria: 1) surface probability, 2) prediction of alpha-helicity by two different methods; 3) prediction of beta-sheet regions by two different methods; 4) prediction of U-turns by two different methods; 5) hydrophilicity/hydrophobicity; and flexibility. The traces of the profiles generated by 30 the computer analyses are shown in Fig. 20. In the hydrophilicity profile, deflection above the abscissa indicates hydrophilicity, and below the abscissa indicates hydrophobicity. The probability that a polypeptide region is antigenic is usually considered to increase when there is a deflection upward from the abscissa in the hydrophilic and/or antigenic profile. It should be noted, however, that these profiles are not necessarily indicators of the strength of the immunogenicity of a polypeptide.

35

Identification of Co-linear Peptides in HCV and Flaviviruses

40 The amino acid sequence of the putative polyprotein encoded in the HCV cDNA sense strand was compared with the known amino acid sequences of several members of Flaviviruses. The comparison shows that homology is slight, but due to the regions in which it is found, it is probably significant. The conserved colinear regions are shown in Fig. 21. The amino acid numbers listed below the sequences represent the number in the putative HCV polyprotein (See Fig. 17.)

45 The spacing of these conserved motifs is similar between the Flaviviruses and HCV, and implies that there is some similarity between HCV and these flaviviral agents.

The following listed materials are on deposit under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC), 12301 Parklawn Dr., Rockville, Maryland 20852, and have been assigned the following Accession Numbers.

50

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EP 0 388 232 A1

lambda-gt11	ATCC No.	Deposit Date
HCV cDNA library	40394	1 Dec. 1987
clone 81	40388	17 Nov. 1987
clone 91	40389	17 Nov. 1987
clone 1-2	40390	17 Nov. 1987
clone 5-1-1	40391	18 Nov. 1987
clone 12f	40514	10 Nov. 1988
clone 35f	40511	10 Nov. 1988
clone 15e	40513	10 Nov. 1988
clone K9-1	40512	10 Nov. 1988
JSC 308	20879	5 May 1988
ps356	67683	29 April 1988

15

In addition, the following deposits were made on 11 May 1989.

Strain	Linkers	ATCC No.
D1210 (Cf1/5-1-1)	EF	67967
D1210 (Cf1/81)	EF	67968
D1210 (Cf1/CA74a)	EF	67969
D1210 (Cf1/35f)	AB	67970
D1210 (Cf1/279a)	EF	67971
D1210 (Cf1/C36)	CD	67972
D1210 (Cf1/13i)	AB	67973
D1210 (Cf1/C33b)	EF	67974
D1210 (Cf1/CA290a)	AB	67975
HB101 (AB24/C100 #3R)		67976

35

The following derivatives of strain D1210 were deposited on 3 May 1989.

Strain Derivative	ATCC No.
pCF1CS/C8f	67956
pCF1AB/C12f	67952
pCF1EF/14c	67949
pCF1EF/15e	67954
pCF1AB/C25c	67958
pCF1EF/C33c	67953
pCF1EF/C33f	67050
pCF1CD/33g	67951
pCF1CD/C39c	67955
pCF1EF/C40b	67957
pCF1EF/CA167b	67959

The following strains were deposited on May 12, 1989.

55

Strain	ATCC No.
Lambda gt11(C35)	40603
Lambda gt10(beta-5a)	40602
D1210 (C40b)	67980
D1210 (M16)	67981

5

10 The deposited materials mentioned herein are intended for convenience only, and are not required to practice the present invention in view of the descriptions herein, and in addition these materials are incorporated herein by reference.

15

Industrial Applicability

20 The invention, in the various manifestations disclosed herein, has many industrial uses, some of which are the following. The HCV cDNAs may be used for the design of probes for the detection of HCV nucleic acids in samples. The probes derived from the cDNAs may be used to detect HCV nucleic acids in, for example, chemical synthetic reactions. They may also be used in screening programs for anti-viral agents, to determine the effect of the agents in inhibiting viral replication in cell culture systems, and animal model systems. The HCV polynucleotide probes are also useful in detecting viral nucleic acids in humans, and thus, may serve as a basis for diagnosis of HCV infections in humans.

25 In addition to the above, the cDNAs provided herein provide information and a means for synthesizing polypeptides containing epitopes of HCV. These polypeptides are useful in detecting antibodies to HCV antigens. A series of immunoassays for HCV infection, based on recombinant polypeptides containing HCV epitopes are described herein, and will find commercial use in diagnosing HCV induced NANBH, in screening blood bank donors for HCV-caused infectious hepatitis, and also for detecting contaminated blood from infectious blood donors. The viral antigens will also have utility in monitoring the efficacy of anti-viral agents in animal model systems. In addition, the polypeptides derived from the HCV cDNAs disclosed herein will have utility as vaccines for treatment of HCV infections.

30 The polypeptides derived from the HCV cDNAs, besides the above stated uses, are also useful for raising anti-HCV antibodies. Thus, they may be used in anti-HCV vaccines. However, the antibodies produced as a result of immunization with the HCV polypeptides are also useful in detecting the presence of viral antigens in samples. Thus, they may be used to assay the production of HCV polypeptides in chemical systems. The anti-HCV antibodies may also be used to monitor the efficacy of anti-viral agents in screening programs where these agents are tested in tissue culture systems. They may also be used for passive immunotherapy, and to diagnose HCV caused NANBH by allowing the detection of viral antigen(s) in both blood donors and recipients. Another important use for anti-HCV antibodies is in affinity chromatography for the purification of virus and viral polypeptides. The purified virus and viral polypeptide preparations may be used in vaccines. However, the purified virus may also be useful for the development of cell culture systems in which HCV replicates.

35 Antisense polynucleotides may be used as inhibitors of viral replication.
40 For convenience, the anti-HCV antibodies and HCV polypeptides, whether natural or recombinant, may be packaged into kits.

50 **Claims**

1. A recombinant polynucleotide comprising a sequence derived from HCV cDNA, wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.
2. A recombinant polynucleotide according to claim 1, encoding an epitope of HCV.
3. A recombinant vector comprising the polynucleotide of claim 1 or claim 2.
4. A host cell transformed with the vector of claim 3.

5. A recombinant expression system comprising an open reading frame (ORF) of DNA derived from the recombinant polynucleotide of claim 1 or claim 2, wherein the ORF is operably linked to a control sequence compatible with a desired host.

6. A cell transformed with the recombinant expression system of claim 5.

5 7. A polypeptide produced by the cell of claim 6.

8. A purified polypeptide comprising an epitope encoded within HCV cDNA wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

9. An immunogenic polypeptide produced by a cell transformed with a recombinant expression vector comprising an ORF of DNA derived from HCV cDNA, wherein the HCV cDNA is comprised of a sequence 10 derived from the HCV cDNA sequence in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the ORF is operably linked to a control sequence compatible with a desired host.

10. A peptide comprising an HCV epitope, wherein the peptide is of the formula

15 AA_x-AA_y,

wherein x and y designate amino acid numbers shown in Fig. 17, and wherein the peptide is selected from the group consisting of AA1-AA25, AA1-AA50, AA1-AA84, AA9-AA177, AA1-AA10, AA5-AA20, AA20-AA25, AA35-AA45, AA45-AA100, AA40-AA90, AA45-AA65, AA65-AA75, AA80-90, AA99-AA120, AA95-AA110, AA105-AA120, AA100-AA150, AA150-AA200, AA155-AA170, AA190-AA210, AA200-AA250, AA220-AA240,

20 AA245-AA265, AA250-AA300, AA290-AA330, AA290-305, AA300-AA350, AA310-AA330, AA350-AA400, AA380-AA395, AA405-AA495, AA400-AA450, AA405-AA415, AA415-AA425, AA425-AA435, AA437-AA582, AA450-AA500, AA440-AA460, AA460-AA470, AA475-AA495, AA500-AA550, AA511-AA690, AA515-AA550, AA550-AA600, AA550-AA625, AA575-AA605, AA585-AA600, AA600-AA650, AA600-AA625, AA635-AA665, AA650-AA700, AA645-AA680, AA700-AA750, AA700-AA725, AA700-AA750, AA725-AA775, AA770-AA790,

25 AA750-AA800, AA800-AA815, AA825-AA850, AA850-AA875, AA800-AA850, AA920-AA990, AA850-AA900, AA920-AA945, AA940-AA965, AA970-AA990, AA950-AA1000, AA1000-AA1060, AA1000-AA1025, AA1000- 30 AA1050, AA1025-AA1040, AA1040-AA1055, AA1075-AA1175, AA1050-AA1200, AA1070-AA1100, AA1100- AA1130, AA1140-AA1165, AA1192-AA1457, AA1195-AA1250, AA1200-AA1225, AA1225-AA1250, AA1250- AA1300, AA1260-AA1310, AA1260-AA1280, AA1266-AA1428, AA1300-AA1350, AA1290-AA1310, AA1310-

35 AA1340, AA1345-AA1405, AA1345-AA1365, AA1350-AA1400, AA1365-AA1380, AA1380-AA1405, AA1400- AA1450, AA1450-AA1500, AA1460-AA1475, AA1475-AA1515, AA1475-AA1500, AA1500-AA1550, AA1500- AA1515, AA1515-AA1550, AA1550-AA1600, AA1545-AA1560, AA1569-AA1931, AA1570-AA1590, AA1595- AA1610, AA1590-AA1650, AA1610-AA1645, AA1650-AA1690, AA1685-AA1770, AA1689-AA1805, AA1690- AA1720, AA1694-AA1735, AA1720-AA1745, AA1745-AA1770, AA1750-AA1800, AA1775-AA1810, AA1795-

40 AA1850, AA1850-AA1900, AA1900-AA1950, AA1900-AA1920, AA1916-AA2021, AA1920-AA1940, AA1949- AA2124, AA1950-AA2000, AA1950-AA1985, AA1980-AA2000, AA2000-AA2050, AA2005-AA2025, AA2020- AA2045, AA2045-AA2100, AA2045-AA2070, AA2054-AA2223, AA2070-AA2100, AA2100-AA2150, AA2150- AA2200, AA2200-AA2250, AA2200-AA2325, AA2250-AA2330, AA2255-AA2270, AA2265-AA2280, AA2280- AA2290, AA2287-AA2385, AA2300-AA2350, AA2290-AA2310, AA2310-AA2330, AA2330-AA2350, AA2350-

45 AA2400, AA2348-AA2464, AA2345-AA2415, AA2345-AA2375, AA2370-AA2410, AA2371-AA2502, AA2400- AA2450, AA2400-AA2425, AA2415-AA2450, AA2445-AA2500, AA2445-AA2475, AA2470-AA2490, AA2500- AA2550, AA2505-AA2540, AA2535-AA2560, AA2550-AA2600, AA2560-AA2580, AA2600-AA2650, AA2605- AA2620, AA2620-AA2650, AA2640-AA2660, AA2650-AA2700, AA2655-AA2670, AA2670-AA2700, AA2700- AA2750, AA2740-AA2760, AA2750-AA2800, AA2755-AA2780, AA2780-AA2830, AA2785-AA2810, AA2796-

50 AA2886, AA2810-AA2825, AA2800-AA2850, AA2850-AA2900, AA2850-AA2865, AA2885-AA2905, AA2900- AA2950, AA2910-AA2930, AA2925-AA2950, AA2945-end(C' terminal).

11. A polypeptide comprised of the peptide of claim 10.

12. An immunogenic polypeptide attached to a solid substrate, wherein the polypeptide is according to 50 claim 7, or claim 8, or claim 9, or claim 10, or claim 11, or wherein the polypeptide is comprised of an epitope encoded within HCV cDNA wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

13. A monoclonal antibody directed against an epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

14. A preparation of purified polyclonal antibodies directed against a polypeptide comprised of an epitope encoded within HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or

clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

15. A polynucleotide probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or from the complement of the HCV cDNA sequence.

16. A kit for analyzing samples for the presence of polynucleotides from HCV comprising a polynucleotide probe containing a nucleotide sequence of about 8 or more nucleotides, wherein the nucleotide sequence is derived from HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, wherein the polynucleotide probe is in a suitable container.

17. A kit for analyzing samples for the presence of an HCV antigen comprising an antibody which reacts immunologically with an HCV antigen, wherein the antigen contains an epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

18. A kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide containing an HCV epitope encoded within HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh.

19. A kit for analyzing samples for the presence of an HCV antibody comprising an antigenic polypeptide expressed from HCV cDNA in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, wherein the antigenic polypeptide is present in a suitable container.

20. A method for detecting HCV nucleic acids in a sample comprising:

(a) reacting nucleic acids of the sample with a polynucleotide probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA sequence is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, and wherein the reacting is under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample,

(b) detecting a polynucleotide duplex which contains the probe, formed in step (a).

21. An immunoassay for detecting an HCV antigen comprising:

(a) incubating a sample suspected of containing an HCV antigen with an antibody directed against an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-antibody complex; and (b) detecting an antibody-antigen complex formed in step (a) which contains the antibody.

22. An immunoassay for detecting antibodies directed against an HCV antigen comprising:

(a) incubating a sample suspected of containing anti-HCV antibodies with an antigen polypeptide containing an epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the incubating is under conditions which allow formation of an antigen-antibody complex; and

(b) detecting an antibody-antigen complex formed in step (a) which contains the antigen polypeptide!

23. An immunoassay for detecting antibodies directed against an HCV antigen comprising:

(a) incubating a sample suspected of containing anti-HCV antibodies with the polypeptide of claim 9, under conditions which allow formation of an antigen-antibody complex; and (b) detecting an antibody-antigen complex formed in step (a) which contains the antigen polypeptide.

24. A vaccine for treatment of HCV infection comprising an immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 or is the sequence present in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

25. A method for producing antibodies to HCV comprising administering to an individual an isolated

immunogenic polypeptide containing an HCV epitope encoded in HCV cDNA, wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, or is of the sequence present in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33C or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, and wherein the immunogenic polypeptide is present in a pharmacologically effective dose in a pharmaceutically acceptable excipient.

- 5 26. An antisense polynucleotide derived from HCV cDNA, wherein the HCV cDNA is that shown in Fig. 17.
- 10 27. A method for preparing purified fusion polypeptide C100-3 comprising:
 - (a) providing a crude cell lysate containing polypeptide C100-3,
 - (b) treating the crude cell lysate with an amount of acetone which causes the polypeptide to precipitate,
 - (c) isolating and solubilizing the precipitated material,
 - (d) isolating the C100-3 polypeptide by anion exchange chromatography, and
 - 15 (e) further isolating the C100-3 polypeptide of step (d) by gel filtration.
- 20 28. A method for preparing an HCV polypeptide comprising:
 - (a) providing a host cell transformed with a recombinant expression system comprising an open reading frame (ORF) of DNA derived from HCV cDNA, wherein the HCV cDNA is in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a; or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17, wherein the ORF is operably linked to a control sequence compatible with a desired host; and
 - (b) incubating the host cell under conditions which allow expression of the HCV polypeptide.
- 25 29. A method for preparing an immunogenic HCV polypeptide comprising:
 - (a) providing a host cell transformed with a recombinant expression vector comprising an ORF of DNA derived from HCV cDNA, wherein the HCV cDNA is comprised of a sequence derived from the HCV cDNA sequence in clone CA279a, or clone CA74a, or clone 13i, or clone CA290a, or clone 33c, or clone 40b, or clone 33b, or clone 25c, or clone 14c, or clone 8f, or clone 33f, or clone 33g, or clone 39c, or clone 15e, wherein the ORF is operably linked to a control sequence compatible with the desired host; and
 - (b) incubating the host cell under conditions which allow expression of the HCV polypeptide.
- 30 30. A method for preparing a host cell transformed with a recombinant polynucleotide comprising a sequence of HCV cDNA derived from the HCV cDNA in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 comprising:
 - (a) providing a host cell capable of transformation;
 - (b) providing the recombinant polynucleotide; and
 - (c) incubating (a) with (b) under conditions which allow transformation of the host cell with the polynucleotide.
- 35 31. A method for preparing a recombinant polynucleotide comprised of a sequence of HCV cDNA derived from the HCV cDNA in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156e, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17 comprising:
 - (a) providing a host cell transformed with the recombinant polynucleotide; and
 - (b) isolating said polynucleotide from said host cell.
- 40 32. A method for preparing blood free of HCV comprising:
 - (a) providing a sample of blood suspected of containing HCV and anti-HCV antibodies;
 - (b) providing an immunogenic polypeptide prepared according to claim 28 or 29;
 - (c) incubating the sample of (a) with the immunogenic polypeptide of (b) under conditions which allow the formation of antibody-HCV polypeptide complexes;
 - (d) detecting the complexes formed in step (c); and
 - (e) saving the blood from which complexes were not detected in (d).
- 45 33. A method for preparing blood free of HCV comprising:
 - (a) providing nucleic acids from a sample of blood suspected of containing HCV polynucleotides;
 - (b) providing a probe for HCV, wherein the probe is comprised of an HCV sequence derived from an HCV cDNA which is of a sequence indicated by nucleotide numbers -319 to 1348 or 8659 to 8866 in Fig. 17.

- (c) reacting (a) with (b) under conditions which allow the formation of a polynucleotide duplex between the probe and the HCV nucleic acid from the sample;
 - (d) detecting a polynucleotide which contains the probe, formed in step (c); and
 - (e) saving the blood from which complexes were not detected in (d).
- 5 34. A method for producing a hybridoma which produces anti-HCV monoclonal antibodies comprising:
- (a) immunizing an individual with an immunogenic polypeptide containing an epitope encoded in HCV cDNA, wherein the HCV cDNA is HCV cDNA in clone 13i, or clone 26j, or clone 59a, or clone 84a, or clone CA156a, or clone 167b, or clone pi14a, or clone CA216a, or clone CA290a, or clone ag30a, or clone 205a, or clone 18g, or clone 16jh, or wherein the HCV cDNA is of a sequence indicated by nucleotide numbers 10 -319 to 1348 or 8659 to 8866 in Fig. 17; or
 - (b) immunizing an individual with an immunogenic polypeptide prepared according to claim 29;
 - (c) immortalizing antibody producing cells from the immunized individual;
 - (d) selecting an immortal cell which produces antibodies which react with an HCV epitope in the immunogenic polypeptide of (a) or (b); and
- 15 (e) growing said immortal cell.

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Translation of DNA 12f

IlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsn
 1 CCATATTAAAATCAGGATGTACGTGGAGGGTCGAACACAGGCTGAAAGCTGCCCTGCA
 GGTATAAATTAGTCCCTACATGCACCCCTCCCCAGCTTGTGTCGGACCTTCGACCGGACGT

TrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeu
 61 ACTGGACGCGGGCGAACGGTGGCATCTGGAAAGACAGGGACAGGTCCGAGCTCAGCCCGT
 TGACCTGCGCCCCGCTGCAACGCTAGACCTCTGTCCCTGTCAGGCTCGAGTCGGCA

LeuLeuThrThrThrGlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeu
 121 TACTGCTGACCACTACACAGTGCGAGGTCCCTCCGTGTTCTTCACAACCCCTACCAGCCT
 ATGACGACTGGTGTGACCTGTCACCGTCCAGGAGGGCACAGGAAGTGTGGGATGGTCGGA

SerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyVal
 181 TGTCCACCGGCGCTCATCCACCTCACAGAACATTGGGACGTGCAGTACTTGTACGGGG
 ACAGGTGGCCGGAGTAGGTGGAGGTGGCTTGTAAACACCTGCACGTCAATGAAACATGCC

GlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeu
 241 TGGGGTCAAGCATCGCGTCTGGGCCATTAAGTGGGAGTACGTCGTTCTCCTGTTCTTC
 ACCCCCAGTTCGTAGCGCAGGACCCGTAATTCAACCTCATGCAGCAAGAGGACAAGGAAG

LeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeuIleSerGlnAlaGlu
 301 TGCTTGAGACGCGCGTCTGCTCCCTGCTTGATGCTACTCATATCCCAAGCGG
 ACGAACGCTCGCGCGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTCGCC

-----Overlap with 141-----
 AlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeu
 361 AGGCAGGCTTGGAGAACCTCGTAATACTTAATGCAAGCATCCCTGGCCGGACGCACGGTC
 TCCGCCGAAACCTCTGGAGCATTAATGAATTACGTCGTAGGGACCGGCCCTGCGTGCCAG

 Val
 421 TTGTATC
 AACATAG

FIGURE 1

Translation of DNA k9-1

1 GluCysProGluArgLeuAlaSerCysArgProLeuThrAspPheAspGlnGlyTrpGly
 CAGGCTGCTTGAGAGGCTAGCCAGCTGCCGACCCCTTACCGATTTGACCAAGGGCTGGG
 GTCCGACAGGACTCTCCGATCGTCGACGGCTGGGAATGGCTAAAATCTGGTCCCACCC

61 ProIleSerTyrAlaAsnGlySerGlyProAspGlnArgProTyrCysTyrPhisTyrPro
 GCCCTATCAGTTATGCCAACGGAAAGCGGCCGACCAGCGCCCTACTGCTGGCACTACC
 CGGGATAGTCAAATACGGTGCCTCGCCGGGCTGGTCGCCGGGATGACGACCGTGATGG

121 ProLysProCysGlyIleValProAlaLysSerValCysGlyProValTyrCysPheThr
 CCCCAAAACCTTGCGGTATTGTGCCCGCAAGAGTGTGTGTCGGTATATTGCTTCA
 GGGGTTTGGAACGCCATAACACGGCCCTCTCACACACACAGGCCATATAACGAAGT

181 ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly
 CTCCCAGCCCCGTGGTGGTGGGAAACGACCGACAGGTGGCGCCACCTACAGCTGGG
 GAGGGTCCGGGCACCACCCCTTGCTGGCTGTCCAGCCCCGGGGTGGATGTCGACCC

241 GluAsnAspThrAspValPheValleuAsnAsnThrArgProProLeuGlyAsnTrpPhe
 GTGAAAATGATACGGACGTCTCGCTTAACAATACCAGGCCACCGCTGGCAATTGGT
 CACTTTACTATGCCAGAAGCAGGAATTGTATGGTCCGGTGGCACCCGTTAACCA

301 GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal
 TCGGGTGTACTGGATGAACTCAACTGGATTCAACAAAGTGTGCGGAGCGCCCTCCTGTG
 AGCCAACATGGACCTACTTGACCTAAGTGGTTTACACCGCTCGGGAGGAACAC

361 IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro
 TCATGGAGGGCGGGCAACAAACACCCCTGCACTGCCCACTGATTGCTTCGCAAGCATC
 AGTAGCCTCCCGCCCGTGTGTGGACGTGACGGGTGACTAACGAAGGCCTCGTAG

421 AspAlaThrTyrSerArgCysGlySerGlyProTrpIleThrProArgCysLeuValAsp
 CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATCACACCCAGGTGCTGGTGC
 GCCTGGTGTATGAGAGGCCACGCCAGGGACCTAGTGTGGTCCACGGACCAGC

481 TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg
 ACTACCCGTATAGGCTTGGCATTATCTGTACCATCAACTACACTATATTAAATCA
 TGATGGGCATATCGAACCGTAATAGGAACATGGTAGTTGATGTGATATAAATTAGT

541 MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu
 GGATGTACGGGGAGGGTGCAGCACAGGCTGGAAAGCTGCCGCAACTGGACGCCGGCG
 CCTACATGCACCCCTCCCCAGCTGTCCGACCTTGACGGACGTTGACCTGCGCCCCCG

601 ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr
 AACGGTGCATCTGGAAAGATAGGGACAGGTCCGAGCTCAGCCCGTACTGCTGACCAACTA
 TTGCAACGCTAGACCTCTATCCCTGTCAGGCTCGAGTCGGCAATGACGACTGGTGAT

661 GlnTrpGlnValLeuProCysSerPheThrLeuProAlaLeuSerThrGlyLeuIle
 CACAGTGGCAGGTCTCCGTGTTCTCACAAACCTGCCAGGCCACGGCCCTCA
 GTGTCACCGTCCAGGAGGGCACAAGGAAGTGTGGACGGTCCGGAACAGGTGGCCGGAGT

721 Overlap with Combined ORF of DNAs 12f through 15e-----
 HisLeuHisGlnAsnIleValAspValGlnTyrLeuTyrGlyValGlySerSerIleAla
 TCCACCTCCACCAGAACATTGTGGACGTGCAGTACTGTACGGGTGGGTCAAGCATCG
 AGGTGGAGGTGGTCTTGTAAACACCTGCACGTCAAGAACATGCCCAACCCAGTTGCTAGC

761 SerTrpAlaIleLysTrpGluTyrValValLeuLeuPheLeuLeuAlaAspAlaArg
 CGTCCTGGGCATTAAGTGGAGTACGTCGCTCTGTTCTCTGCTTGACGCCCG
 CGAGGACCCGGTAATTACCCCTCATGCAGCAGGAGGACAAGGAAGACGAACGTCTGGCG

FIGURE 2-1

841 ValCysSerCysLeuTrpMetValLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsn
 GCGTCTGCTCCCTGCTTGATGCTACTCATATCCCAGCGGAAGCGGTTGGAGA
 CGCAGACGAGGACGAACACCTACTACGATGAGTATAGGGTTGCCCTCGCCGAAACCTCT

901 LeuValIleLeuAsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuVal
 ACCTCGTAATACTTAATGCAGCATTCCCTGGCCGGACGCACGGCTTGTATCCCTCCG
 TGGAGCATTATGAATTACGTCGTAGGGACCAGGCCCTCGCTGCCAGAACATAGGAAGGAGC

961 PhePheCysPheAlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPhe
 TGTTCTCTGCTTGCACTGGTATCTGAAGGGTAAGTGGTGCCTGGAGCGGTCTACACCT
 ACAAGAACGAAACGTACCATAGACTTCCCATTCAACCCACGGGCTGCCAGATGTTGA

1021 TyrGlyMetTrpProLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeu
 TCTACGGGATGTCGGCTCTCCCTGCTCTGTTGGCTTGCCCCAGCGGCGTACGGCG
 AGATGCCCTACACCGGAGGGAGGACGAGGACAACCGCACGGGGTCCGGCATGGCG

1081 AspThrGluValAlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThr
 TGGACACGGAGGTGGCCGCGTCGTGGCGGTTGTTCTCGCGGGTTGATGGCGCTAA
 ACCTGTGCCCTCACCGGCGCAGCACACGCCACAAGAGCAGCCCCTACCGCGATT

1141 LeuSerProTyrTyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeu
 CTCTGTCACCAATTACAAGCGCTATATCAGCTGGTGTGGCTTGGCTTCAGTATTTTC
 GAGACAGTGGTATAATGTTCGCGATATAGTCGACCACGAAACACCACCGAAGTCATAAAAG

1201 ThrArgValGluAlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyArg
 TGACCAAGAGTGGAAAGCGCAACTGCACGTGTGGATTCCCCCCTCAACGTCCGAGGGGGC
 ACTGGTCTACCTTCGCGTTGACGTGCACACCTAAGGGGGGAGTTGCAGGCTCCCCCG

1261 AspAlaValIleLeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLys
 GCGACGCTGTCATCTTACTCATGTTGCTGTACACCCGACTCTGGTATTGACATACCA
 CGCTGCGACAGTAGAAATGAGTACACACGACATGGGGCTGAGACCATAAACTGTAGTGGT

1321 LeuLeuLeuAlaValPheGlyProLeuTrpIleLeuGlnAla
 AATTGCTGCTGGCCGTCCTCGGACCCCTTGGATTCTCAAGCCAG
 TTAACGACGACCGGAGAACCTAAGAAGTTCGGGT

FIGURE 2-2

Translation of DNA 15e

GlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAla
 1 CGCGCTGGAAAGAGGGTTACTACCTCACCCGTGACCTACAAACCCCCCTCGCAGAGC
 GCCGCGACCTTCCTCCCAGATGATGGAGTGGGACTGGGATGTTGGGGAGCGCTCTG
 -----Overlap with 26g-----
 61 AlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPhe
 TGCCTGGGAGACAGCAAGACACACTCCAGTCATTCTGGCTAGGCAACATAATCATGTT
 ACGACCCCTCTGTCGTTCTGTGAGGTCAGTTAAGGACCGATCCGTTGTATTAGTACAA

 121 AlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAla
 TGCCCCCACACTGTGGCGAGGATGATACTGATGACCCATTCTTAGCGTCCTTATAGC
 ACGGGGGTGTGACACCCGCTCTACTATGACTACTGGTAAAGAAATCGCAGGAATATCG

 181 ArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGln
 CAGGGACCAGCTTGAAACAGGCCCTCGATTGGAGATCTACGGGCCTGCTACTCCATAGA
 GTCCCTGGTCGAACCTGTCCGGAGETAACGCTCTAGATGCCCGACGATGAGGTATCT

 241 ProLeuAspLeuProProIleIleGlnArgLeu
 ACCACTTGATCTACCTCCAATCATTCAAAGACTC
 TGGTGAACTAGATGGAGGTAGTAAGTTCTGAG

FIGURE 3

Translation of DNA 131

ProSerProValValValGlyThrThrAspArgSerGlyAlaProThrTyrSerTrpGly
 1 CTCCCAGCCCCGGGGTGGGAAACGACCGACAGGTGGCGGCCCTACCTACAGCTGGG
 GAGGGTCGGGGCACCACCAACCCCTGGCTGGCTGCCAGCCCGCGGGATGGATGTCGACCC

GluAsnAspThrAspValPheValLeuAsnAsnThrArgProProLeuGlyAsnTrpPhe
 61 GTGAAAATGATAACGGACGTCTCGTCTTAACAATACCAAGGCCACCGCTGGGCAATTGGT
 CACTTTTACTATGCCTGCAGAACGAGGAATTGGTATGGTCCGGTGGCGACCCGTTAACCA

GlyCysThrTrpMetAsnSerThrGlyPheThrLysValCysGlyAlaProProCysVal
 121 TCGGTTGTACCTGGATGAACTCAACTGGATTCAACCAAAGTGTCGGAGCGCCTCCTTG
 AGCCAACATGGACCTACTTGAGTTGACCTAAGTGGTTCACACGCCCTCGCGGAGGAACAC

IleGlyGlyAlaGlyAsnAsnThrLeuHisCysProThrAspCysPheArgLysHisPro
 181 TCATCGGAGGGGCGGGCAACAAACACCCCTGCACTGCCCACTGATTGCTTCCGCAAGCATC
 AGTAGCCTCCCCGCCGTTGTTGGGACGTGACGGGGTGAACTAACGAAGGCGTCTCGTAG

AspAlaThrTyrSerArgCysGlySerGlyProTrpLeuThrProArgCysLeuValAsp
 241 CGGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGCTCACACCCAGGTGCCCTGGTC
 GCCTGCGGTGTATGAGAGGCCACGCCAGGGACCGAGTGTGGGTCCACGGACCCAGC

TyrProTyrArgLeuTrpHisTyrProCysThrIleAsnTyrThrIlePheLysIleArg
 301 ACTACCCGTATAGGCTTGGCATTTATCCTGTACCATCAACTACACCCATATTTAAAAATCA
 TGATGGGATATCCGAAACCGTAATAGGAACATGGTAGTGTGATGTGTATAAATTAGT

MetTyrValGlyGlyValGluHisArgLeuGluAlaAlaCysAsnTrpThrArgGlyGlu
 361 GGATGTACGTGGGAGGGTCGAGCACAGGCTGGAAGCTGCCCTGCAACTGGACGCCGGCG
 CCTACATGCAACCTCCCCAGCTGTGTCGCCACCTCGACGGACGTTGACCTGCCCGC

-----Overlap with 12f-----

ArgCysAspLeuGluAspArgAspArgSerGluLeuSerProLeuLeuLeuThrThrThr
 421 AACGTTGCGATCTGGAAGACAGGGACAGGTCCGAGCTCAGCCCGTTACTGCTGACCACTA
 TTGCAACCGCTAGACCTCTGTCCCTGTCCAGGCTCGAGTCGGGCAATGACGACTGGTGAT

GlnTrpGlnValLeuProCysSerPheThrThrLeuProAlaLeuSerThrGlyLeu
 481 CACAGTGGCAGGTCCCTCCGTGTTCTTCACAACCCCTGCCAGCCTGTCCACCGGCCTCA
 GTGTCACCGTCCAGGAGGGACAAGGAAGTGTGGGACGGTCGGAACAGGTGGCCGGAGT

FIGURE 4

Translation of DNA 26j

1 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
 GCTTTCTATCACCAAGTTCAACTCTTCAGGCTGTCCCTGAGAGGCTAGCCAGCTGCCG
 CGAAAAGATAGTGGTGTCAAGTGAGAAGTCCGACAGGACTCTCCGATCGGTGACGGC

 61 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
 ACCCCTTACCGATTGACCAGGGCTGGGGCCCTATCAGTTATGCCAACGGAAAGCGGCC
 TGGGGAATGGCTAAAATGGTCCCCGACCCCGGGATAGTCAATAACGGTTGCCCTCGCCGG

 121 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
 CGACCAGCGCCCTACTGCTGGCACTACCCCCAAAACCTTGCGGTATTGTGCCCGCAA
 GCTGGTCGCAGGGATGACGACCGTGATGGGGGTTTGGAACGCCATAACACGGCGCTT

 ---Overlap with 131---
 181 SerValCysGlyProValTyrCysPheThrProSerProValValVal
 GAGTGTGTGTGGTCCGGTATATTGCTTCCTGCACTCCAGCCCCGTGGTGGTGG
 CTCACACACACCAGGCCATATAACGAAGTGAGGGTGGGGCACCACCC

FIGURE 5

Translation of DNA CA59a

1 LeuValMetAlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAla
 TTGGTAATGGCTCAGCTGCTCCGGATCCCACAAGCCATCTGGACATGATCGCTGGTGCT
 AACCATTACCGAGTCGACGAGGCCTAGGGTGTGGTAGAACCTGTACTAGCGACCACGA

 61 HisTrpGlyValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysVal
 CACTGGGGAGTCCTGGCGGGCATAGCGTATTCTCATGGTGGGAACTGGGCGAAGGTC
 GTGACCCCTCAGGACCGCCGTATCGATAAAGAGGTACCACCCCTTGACCCGCTTCCAG

 121 LeuValValLeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySer
 CTGGTAGTGTGCTGCTATTGCGGGCGTCGACCGCGAAACCCACGTCACCGGGGGAAAGT
 GACCATCACGACGACGATAAACGGCCGCAGCTGCGCCTTGGGTGCAGTGGCCCCCTTCA

 181 AlaGlyHisThrValSerGlyPheValSerLeuLeuAlaProGlyAlaLysGlnAsnVal
 GCCGGGCCACACTGTGTCGGATTGGTTAGCCTCTCGCACCGCGCCAAGCAGAACGTC
 CGGCCGGTGTGACACAGACCTAAACAATCGGAGGAGCGTGGTCCCGCGGTTCTGCAG

 241 GlnLeuIleAsnThrAsnGlySerTrpHisLeuAsnSerThrAlaLeuAsnCysAsnAsp
 CAGCTGATCAAACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGAAGTCAATGAT
 GTCGACTAGTTGTGGTTGCCGTCAACCGTGGAGTTATCGTGCCTGGACTTGACGTTACTA

 301 SerLeuAsnThrGlyTrpLeuAlaGlyLeuPheTyrHisHisLysPheAsnSerSerGly
 AGCCTCAAACCCGGCTGGTTGGCAGGGCTTTCTATCACCAAGTTCAACTCTTCAGGC
 TCGGAGTTGTGGCCGACCAACCGTCCCAGAAAGATAAGTGGTGTCAAGTTGAGAAGTCCG
 -----Overlap with 26j-----

-----Overlap with K9-1-----

361 CysProGluArgLeuAlaSerCysArgPro
 TGTCCTGAGAGGGCTAGCCAGCTGCCGACCC
 ACAGGACTGCCGATCGTCGACGGCTGGGG

FIGURE 6

Translation of DNA CA84a

1 GlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrpAsp
 CGCAAGGTGCAATTGCTCTATCTATCCGGCATATAACGGTCACCGCATGGCATGGG
 GCGTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGGCGTACCGTACCC

61 MetMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIlePro
 ATATGATGATGAACTGGTCCCCTACGACGGCGTTGGTAATGGCTCAGCTGCTCCGGATCC
 TATACTACTTGTGACCAGGGGATGCTGCCGCAACCATTACCGAGTCGACGAGGCCCTAGG

121 GlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAlaTyr
 CACAAGCCATCTGGACATGATCGCTGGTGCTCACTGGGGAGTCCTGGCGGGCATAGCGT
 GTGTTGGTAGAACCTGTACTAGCGACCACGAGTGACCCCTCAGGACCGCCCCGTATCGCA

Overlap with CA59a

181 PheSerMetValGlyAsnTrpAlaLysValLeuValLeuLeuLeuPheAlaGlyVal
 ATTTCTCCATGGTGGGGAACTGGCGAAGGTCTGGTAGTGCTGCTGCTATTGCCGGCG
 TAAAGAGGTACCACCCCTTGACCCGCTCCAGGACCATCACGACGATAAACGGCCGC

241 AspAlaGluThrHisValThrGly
 TCGACGCCGGAAACCCACGTACCGGGGG
 AGCTGCGCTTGGGTGCAGTGGCCCC

FIGURE 7

Translation of DNA CA156e

1 CysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGln
 GTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACTCCCCGCGACGCA
 CACAACCCACCGCTACTGGGGATGCCACCGGTGGTCCCTACCGTTGAGGGGGCGCTGCCT

 61 LeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrVal
 GCTTCGACGTCACATCGATCTGCTTGTGGGAGCGCCACCTCTGTTCGGCCCTCTACGT
 CGAAGCTGCAGTAGCTAGACGAACAGCCCTCGCGGTGGAGACAAGCCGGAGATGCA

 121 GlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArg
 GGGGGACCTATGCGGGTCTGTCTTCTGTGGCCAAGTGTACCTCTCTCCAGGCG
 CCCCCTGGATA CGCCCAGACAGAAAGAACAGCCGGTGACAAGTGGAAAGAGAGGGTCCGC

 181 HisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArg
 CCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCCAGGCCATAAACGGGTACCG
 GGTGACCTGCTGCCTTCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCAGTGGC

 -----Overlap with CA84a-----
 241 MetAlaTrpAspMetMetAsnTrpSerProThrThrAlaLeuValValAlaGlnLeu
 CATGGCATGGGATATGATGATGAACTGGTCCCTACGACGGCGTTGGTAGTGGCTCAGCT
 GTACCGTACCCCTATACTACTACTTGTGACCAGGGATGCTGCCGCAACCACCAACCGAGTCGA

 301 LeuArgIleProGlnAla
 GCTCCGGATCCCACAAGCC
 CGAGGCCTAGGGTGGTCCG

FIGURE 8

Translation of DNA CA157b

1 SerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAla
 CTCCACGGGGCTTTACCAACGTCACCAATGATTGCCCTAACCGAGTATTGTGTACGAGGC
 GAGGTGCCCCGAAATGGTGCAGTGGTTACTAACGGGATTGAGCTCATAACACATGCTCCG
 AlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSer
 61 GGCCGATGCCATCCTGCACACTCCGGGGTGCCTTGCGTTGAGGGCAACGCCCTC
 CCGGCTACGGTAGGACGTGTGAGGCCAACGCAAGGAAACGCAACTCCGTTGCGGAG
 ArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThr
 121 GAGGTGTTGGGTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAACCTCCCGCGAC
 CTCCACAAACCCACCGCTACTGGGGATGCCACCAGGTGGTCCCTACCGTTGAGGGCGCTG
 -----Overlap with CA156e-----
 GlnLeuArgArgHisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyr
 181 GCAGCTTCGACGTCACATCGATCTGCTTGTGCGGGAGCGCTACCCCTCTGTTGCGCCCTCTA
 CGTCAAGCTGAGTGTAGCTAGACGAACAGCCCTCGCGATGGGAGACAAGCCGGGAGAT

 ValGlyAspLeuCysGlySerValPheLeu
 241 CGTGGGGGACTTGTGCGGGCTGTCTTCTTG
 GCACCCCTGAACACGCCAACAGAAAC

FIGURE 9

Translation of DNA CA216a

1 LeuIArgArgSerArgAsnLeuGlyLysValIleAspThrLeuIleCysGlyPheAlaAsp
 CCGCGGCTTACGGTCCGGCAATTGGGTAAAGTCATCGATAACCTTACGTGCGGCTTCGCCG
 GGGCGCATCCAGCCGTTAACGCCATTCCAGTAGCTATGGAAATGCCACCCCCAACCGCC

 61 LeuMetGlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAla
 ACCTCATTCGGCTACATACCCCTCTCGGCGCCCGCTTGGAGGGCGCTGCCAGGGCCCTGG
 TGAGTACCCATGTATGGCGAGCMCGCGGGGAGAACCTCCCGCACGGTCCCCGGGACC

 121 HisGlyValArgValLeuGluAspGlyValAspTyrAlaIleGlyAlaAspLysProGlyCys
 CGCATGGCGTCCGGGTTCTGGAGAOGGCGTGAACTATGCAACAGGGAACTTCCCTGGCTT
 CGGTACCCCGCCCGCCAGACCTTCGCGCACTTGAAACCGTTGCTCGTGGAGAACCGAA

 181 SerPheAspIlePheLeuIleAlaIleAspCysLeuThrValProAlaSerAlaTyr
 GCTCTTCTCATCTTCTCTGGCCCTGCTCTTGCTTGACTGTGCCCCGCTTCGGCTT
 CGAGAAAGAGATAGAAGGAAGACCGGGACCAGAACGAACTGACACCCCCCGAACCGGA

 241 GlyValArgAsnSerThrGlyLeuTyrHisValThrAspAspCysProAsnSerSerIle
 ACCAGTGCGCAACTCCACGGGGCTTACCAACGTCACCAATGATTGCCCTAACTCCAGTA
 TGGTTACCGCGTTGAGGTGCCCGAAATGGTGCAGTGTTACTAACGGGATTGAGCTCAT

 Overlap with CA167b

 301 ValTyrGluAlaIleAspAlaIleLeuHistThrProGlyCysValProCysValArgGlu
 TTGTCTACCAAGCGCCCGATGCCATCTGCACACTCCGGGGTGCCTCCCTTGCGTGG
 AACACATGCTTGGCGGCTACGGTAGGACGTGTGAGGCCAACGCAGGGAAACGCAACGAC

 361 GlyAsnAlaSerArgCysTrpValAlaMetThrProThrValAla
 AGGCACACCGCTCGAGGTGTTGGGTGGCGATGACCCCTACGGTGGCC
 TCCCGTTGGGAGCTCCACAACCCACCGCTACTGGCAATCCACCGC

FIGURE 10

Translation of DNA CA239ca

1 LysLysAsnLysArgAsnThrAsnArgArgPheGlnAspValLysPheProGlyGlyGly
 AAAA.....ACAAACGTAACACCAACCGTCGCCACAGGACSTCAAGTTCCCGGGTGGCG
 TTTTTTTTTGTGTTGCATIGTGCTTGGCAGCGGGTGTCTCTGCAACTCAAGGGCCCACCGC

 61 GluIleValGlyGlyValItyLeuLeuPheArgArgGlyPheArgLeuGlyValArgAla
 GTCAAGATGTTGGCTGGAGTTTACTTGTGCGGGCAGGGGGCTTAGATTGGGTGGCG
 CAGTCTAGCAACCCACCTCAAATGAACAAACGGCGCTCCCCGGGATCTAACCCACACGCG

 121 ThrArgLysThrSerGluArgSerGlnPheArgArgGlyArgArgGlnPheProLysAla
 CGACCGACAAAGACTTCCGACCCGCTCCAAACCTCCACCTAGACCCGACCCCTATCCCCAAC
 GCTGCTCTTCTGAGGCTGCCAACGGTGGAGCTCCATCTGGGGTCGGATAGGGGTTCC

 181 ArgArgPheGluGlyArgThrTrpAlaGlnPheGlyTyrPhePheLeuItyGlyAsn
 CTCGTCGGCCCGAGGGCAGGACCTGGCTCAGCCCGGGTACCTTGGCCCTCTATGGCA
 GACCACCGGGCTCCCCCTCCGACCCGACTCCCCCCTACGGAAACCGGGCACATACCG

 241 GluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPheArgGlySerArgPheSerTrpGly
 ATGAGGGCTCGGGTGGCGATGGCTCCCTGCTCCCCGATGGCTCTGGGCTAGCTGGG
 TACTCCCCACCCGACCCGCTACCCGACACGACCCCCCACCGAGAGCCGGATCGACCC

 301 ProThrAspPheArgArgArgSerArgAsnLeuGlyLysValLysAspPheLeuThrCys
 GCCCCCACAGACCCGGCTAGTCGGCAATTGGCTAAGGTATCGATAACCTTACGT
 CGGGGTGTCGGGGGGGGCATCCAGCGGTTAACCCATTCCAGTAGCTATGGCAATCCA

 361 GlyPheAlaAspLeuMetGlyTyrIlePheLeuValGlyAlaProLeuGlyGlyAlaAla
 CCCCCCTGGGGGACCTCATGGCTACATACCGCTCTCCCTCCCCCTCTGGGAGCCGGCTG
 CGCCGAAACGGGCTGGAGTACCCCATGTATGGCGAGCAGCCGCGGGAGAACCTCCGGGAC

 Overlap with CA216a-----
 421 ArgAlaLeuAlaHisGlyValArgValLeuGlyAspGlyValAspTyrAlaThrGlyAsn
 CCAGGGCCCTGGGGCATGGCTCCCCCTCTGGAAAGACGGGCTGAACTATGCAACAGCG
 GGCTCCCCGGACCCGCTACCCGAGGCCAAGACCTCTGGCGACTTGTGATACTGGTCCCT

 481 LeuProGlyCysSerPheAspThrPhe
 ACCTTCCCTGGCTCTTCTCTACCCCT
 TGGAGGACCAACGAGAAAAGAGATGGAG

FIGURE 11

→ Translation of DNA ag30a

#MetSerValValGlnProProGlyProProLeu

1 OCCACRAAGCGCTCTAGCCATGGCCTTAC TATGACTGTGCGTGCAGGCCCTCCAGGACCCCCCCC
 GCGTCATTGGAGATCGGTACCGCAATCTACTCACAGCACGTGGAGGTCTTGGGGGGGG

ProGlyGluProM

61 TCCCGGGAGAGCCATACTGCGTCGGAAACCGGTGAGTACACCGGAATGCCAGGACGGAC
 AGGGCCCTCTGGTATCACCAGACGCCCTGGCCACTATGTGGCCTAACGGTCTGCTG

#MetPheGlyAspIleGlyValProProGlnAsp

121 CGGTCCCTTCITGGATCAACCCGCTCAAATGCCCTGGAGATTGGGGGTGCCCCCGCMAGA
 GCCCAGGAAAGAACCTAGTTGGCGAGTACGGACCTCTAAACCCGACGGGGCGTTCT

Cys:AM

OP AM GlyAlaCys

181 CTGCTAGCCGAGTAGTGTGGGTGGCAGAACGGCTTGTGGTACTGCCCTAGGGTGCTT
 GACGATCGGCTCATCACAAACCCAGGGCTTCCGGAACACCATCACUGACTATCCCACCAA

GluCys?ProGlyArgSerArgArgProCysThrMetSerThrAsnProLys?ProGlnLys

241 GCGAGTCCCCGGGAGGTCTCGTAGACCGTGACCATGAGCACGAATCTAAACCTCAAA
 CGCTCACGGGGCCCTCCAGACCCTGCGCACTGGTACTCGTGCTTACGATTGGACTTT

LysAsnLysArgAsnThrAsnArgArgProGlnAspValLysPheProGlyGlyGln

301 AAAAAAAACAAACGTAACACCAACCGTGGCCACAGGACGTCAAGTTCCGGGTGGGGTC
 TTTTTTTCTTCATTCCTGGTGGCACCCGGTCTCTCCAGTTCAAGGGCCACCCCGAG

IleValGlyGlyValTyrIleLeuProArgArgGlyProArgLeuGlyValArgAlaThr

361 AGATCGTTGGTGGAGTTACTTGTGGCCCGCAGGGGCCCTAGATGGGTGTGCGCGCGA
 TCTACCAACCACCTCAAATGAAACAACGGCCGCTCCCCGGATCTAAACCCACACGGCCGCT

ArgLysThrSerGluArgSerGlnProArgGlyArgArgGlnProIleProLysAlaArg

421 CGAGAAAAGACTTCCGAGCGGTGCGAACCTCGAGGTAGACGTCAGCCTATCCCGAAGGCTC
 CCTCTTTCTCAAGCTCGCCAGGCTTGGAGCTCCATCTGCACTCGATAGGGCTTCCGAG

ArgProGluGlyArgThrTrpAlaGlnProGlyTyr?ProTrpProLeuTyrGlyAsnGlu

481 Overlap with CA290a-----
 GTCCGGCCCGAGGGCAGGACCTGGGCTCAGCCCCGGTACCCCTGGCCCCCTCTATGGCAATG
 CAGCCGGCTCCCGTCCCTGGACCCGACTCGGGCCCATGGAAACGGGGAGATAACCGTTAC

CysCysAlaPheGlyIleLeuSerProArgGlySerArgProSerTrpGlyPro

541 ~~AGGGCTACCGGTGGCGGGATGGCTCCCTGCTCCCCGTGGCTCTCCGCCTACCTGGGGCG~~
~~TCCCCGAGGCCACCCGCCCTACCGACGAGGGGCACCGAGAGCCGGATCGACCCCCGG~~

ThrAspProArgArgArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGly

601 ~~CCACAGACCCCCGGCGTAGGICGCGCAATTGGGTAAGCTCATCGATACCCCTAACGTGG~~
~~GGTGTCTCCCCGCCATCCAGCGCTAAACCAATTCCAGTAGCTATGGGAATGCACGC~~

Phe

661 GCTTC
CGAAG

- * - Start of long HCV ORF
- | - Putative first amino acid of large HCV polyprotein
- | - Putative small encoded peptides(that may play a translational regulatory role)

FIGURE 12-2

Translation of DNA CA205a

1 ValLeuGlyArgGlnIleArgProCysGlyThrAlaOP AM GlyAlaCysGluCysProGly
 1 GTCCTGGGTGGCGAAAGGCCTTGTTACTGGCTGAAGGGTCTTGGCGAGTCCCCGGG
 CAGAACCCUAGCGCTTCCGGAAACCCATGACGGACTATCCACGAAACGCTCACGGGGCCC

61 ArgSerArgArgProCysThrMetSerThrAsnProLysProGlnArgLysThrLysArg
 61 AGGCTCGTAGACCCTGCAACGACGAATCTAAACCTAAAGAAAAACCAAAACGT
 TCCAGACCATCTGGCACGTGGTACTCTCTTAGGATTGGGTTTCTTTGGTTGCA

121 AsnThrAsnArgArgProGlnAspValLysPhePheArgLysGlyGlnIleValGlyGly
 121 AACACCAACCGTCGCCAACAGGACGTCAAGTCCCGGTTGGCGTCAGATCGTTGGTGGA
 TTGTGCTTGGCACCCGCTCTCCCGCTTCACGGCCCAACGGCCAGTCACGAAACACCT

181 ValTyrLeuLeuProArgArgGlyProArgIleGlyValArgAlaThrArgLysThrSer
 181 GTTTACTTGTGCCCGCAGGGGCCCTAGATGGGTTGGCCGCCACGACAAACACTTCC
 CAAATGAACAAAGGGCGCTCCCGGAACTAACCCACACCGCGACTGCTCTTCTGAAGG

Overlap with CA290a

241 GluArgSerGlnProArgArgGlyArgArgGlnProIleProLysAlaArgArgProGlnArgGly
 241 GACCGCTGGCTAACCTGACCTACACCTCACCTATCCCACCCCTGCTGGCCCGAACCGC
 CTCGCCACGCTTGAGGCTCCATCTGCACTGCGATAAGGGTTGGAGCAGCCGGCTCCCG

301 ArgThrTrpAlaGlnIleProGlyTyrProTrpProLeuTyriArgAspGluGlyCys
 301 AGGACCTGGCTCAACCCGGTACCCCTGGCCCTCTATCCCAATGACGGCTCC
 TCCTGGACCGAGTGGGGCCATGGGAAACGGGGMATAACGGTTACTCCCGAGC

* - putative initiator methionine codon

FIGURE 13

Translation of DNA 18g

#ProProOP
 *SerThrMetAsnHisSerProValArgAsnTyrCysLeuHisAlaGluSerValAla
 *LeuHisAlaGluSerLeuProCysGluGluLeuSerSerArgArgLysArgLeuAla
 1 CTGGCACCATGAACTCTCTCTGTGAGGAACATCTGCTTCACGCCGAAAGCGCTAGCC
 GAGGTGTTACTTACTGAGGGACACTCTTGATGACAGAAGTGGGTCTTCGAGATCGG

 #MetSerValValGlnProProGlyProProLeuProGlyGluProAM

MetAlaLeuValCP
 61 ATGGCGTTAGTATGAGTGTGCGCAGCCCTCCAGGACCCCCCCCCTCCCGGGAGAGCCATAGT
 TACCGCAATCATACTCACAGCACGTGGAGGTCTGGGGGAGGCCCCCTCTGGTATCA

121 GGTCTGCGGAAACCGGTGAGTACACCGGAATTGCCAGGACGGACCCGGTCCCTTCTGGATC
 CCAGACGCCTTGGCACTCATGTCGGCTTAACGGTCTGCTGGCCCAGGAAAGAACCTAG

Overlap with ag30a-----

#MetProGlyAspLeuGlyValProProGlnAspCysAM

181 AACCCGCTCAAAGCCCTGGAGAATTGGGCGTGCCCCCGCAAGACTGCTAGCCGAGTAGTGT
 TTGGCCCACTTACGGACCTCTAAACCCCCACCCCCCCCTCTGACGATCGGCTCATCACA

 OP AM GlyAlaCysGluCysProGlyArgSer

241 TGGGTCGCAGAACGGCTTGTGGTACTGCCTGATAGGGTGTGCGAGTGCCCCGGAGGT
 ACCCAGCGCTTCCGGAACACCAATGACGGACTATCCCACGAACGCTCACGGGGCCCTCCA

ArgArg

301 CTCGTAGA
 GACCATCT

- - Start of long HCV ORF
- § - Putative small encoded peptides(that may play a translational regulatory role).

FIGURE 14

Translation of DNA 16jh

Overlap with 15e

GlyAlaCysTyrSerIleGluProLeuAspLeuProProIleIleGlnArgLeuHisGly
 1 GGGCCCTGCTACTCCATAGAACCACTGGATCTACCTCCAATCATTCAAAGACTCCATGGC
 CCCCGGACGATGAGGTATCTGGTGACCTAGATGGAGGTAGTAAGTTCTGAGGTACCG

 LeuSerAlaPheSerLeuHisSerTyrSerProGlyGluIleAsnArgValAlaAlaCys
 61 CTCAGCGCATTTCACTCCACAGTTACTCTCCAGGTAAATTAAATAGGGTGGCCGCATGC
 GAGTCGCGTAAAGTGAGGTGTCAATGAGAGGTCCACTTTAATTATCCCACCGGCGTACG

 Gly*
 G
 LeuArgLysLeuGlyValProProLeuArgAlaTrpArgHisArgAlaArgSerValArg
 121 CTCAGAAAACCTGGGGTACCGCCCTGCGAGCTGGAGACACCAGGGCCGGAGCGTCCGC
 GAGTCAGCTTTGAACCCCATGGCGGAACGCTCGAACCTCTGTGGCCCGGGCTCGCAGGCG

 AlaArgLeuLeuAlaArgGlyGlyArgAlaAlaIleCysGlyLysTyrLeuPheAsnTrp
 181 GCTAGGCTTCTGGCCAGAGGAGGCAGGGCTGCCATATGTGGCAAGTACCTCTTCAACTGG
 CGATCCGAAGACCGGTCTCCTCCGTCCGACGGTATAACACCGTTATGGAGAAGTTGACC

 AlaValArgThrLysLeuLys
 241 GCAGTAAGAACAAAGCTCAAAC
 CGTCATTCTTGTTCGAGTTG

* - nucleotide heterogeneity

FIGURE 15

COMBINED ORF OF DNAs pil4a THROUGH 15e

(pil4a/CA157b/CA156e/CA84a/CA59a/K9-1/12f/14i/11b/7f/7e/
 8h/33c/40b/37b/35/36/81/32/33b/25c/14c/8f/33f/33g/39c/
 35f/19g/26g & 15e)

1 ArgSerArgAsnLeuGlyLysValIleAspThrLeuThrCysGlyPheAlaAspLeuMet
 AGGTGCGCRAATTGGTAAAGGTATCGATACCTTACGTGCGGCTCGCCGACCTCATG
 TCCAGCGCTTAAACCCATTCCAGTAGCTATGGGAATGCACGCCAAGCGGCTGGAGTAC
 GlyTyrIleProLeuValGlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGly
 61 GGGTACATACCGCTCGTCGGCGCCCTCTGGAGGGCGCTGCCAGGGCTGGCGATGGC
 CCCATGTATGGCGAGCACCCGCGGGAGAACCTCCGCACGGTCCCAGGACCGCGTACCG
 ValArgValLeuGluAspGlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPhe
 121 GTCCGGGTTCTGGAAAGACGGCGTGAACATGCAACAGGGAACCTTCTGGTGTGCTCTTC
 CAGGCCAAGACCTCTGCCGACTTGATACTGGTGTCCCTTGGAAAGGACCAACGAGAAAG
 SerIlePheLeuLeuAlaLeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnVal
 181 TCTATCTCTCTGGCCCTGCTCTTGACTGTGCCGCTCGGCCTACCAAGTG
 AGATAGAAGGAAGACCGGGACGAGAGAACGAACTGACACGGCGAAGCGGATGGTAC
 ArgAsnSerThrGlyLeuTyrHisValThrAsnAspCysProAsnSerSerIleValTyr
 241 CGCAACTCCACGGGGCTTACACGTCACCAATGATTGCCCTAACTCGAGTATTGTGAC
 CGGTGAGGTGCCCGAAATGGTGCACTGGTTACTAACGGGATTGAGCTATAACACATG
 GluAlaAlaAspAlaIleLeuHisThrProGlyCysValProCysValArgGluGlyAsn
 301 GAGGCGGCCGATGCCATCTGCACACTCCGGGTGCGTCCCTTGCGTTGAGGGCAC
 CTCCGCCGCTACGGTAGGTGAGGCCACGCAGGGAACGCAAGCACTCCGTTG
 AlaSerArgCysTrpValAlaMetThrProThrValAlaThrArgAspGlyLysLeuPro
 361 GCCTCGAGGTGTTGGGTTGGCGATGACCCCTACGGTGGCCACCAGGGATGGCAAAC
 CGGAGCTCCACAACCCACCGCTACTGGGGATGCCACCGGTGTCCTACCGTTGAGGG
 AlaThrGlnLeuArgArgHisIleAspLeuValGlySerAlaThrLeuCysSerAla
 421 GCGACGCAGCTTCGACGTACATCGATCTGCTTGCGGAGCGCCACCCCTGTCGCC
 CGCTCGTCAAGCTGCACTGGTAGCTAGACGAACAGCCCTCGCGTGGAGACAGCCGG
 LeuTyrValGlyAspLeuCysGlySerValPheLeuValGlyGlnLeuPheThrPheSer
 481 CTCTACGTGGGGACCTATGGGGCTGCTTTCTGGCCAACTGTTACCTCTCT
 GAGATGCACCCCCTGGATACGCCAGACAGAAACAGCCGTGACAAGTGGAAAGAGA
 ProArgArgHisTrpThrThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThr
 541 CCCAGCGCCACTGGACGACGCAAGGTTGCAATTGCTCTATCTATCCGCCATATAACG
 GGGTCCCGGTGACCTGCTGCGTCCACGTTAACGAGATAGATAGGGCCGGTATATTG
 GlyHisArgMetAlaTrpAspMetMetAsnTrpSerProThrThrAlaLeuValMet
 601 GGTCACCGCATGGCATGGGATATGATGATGAACGGTCCCCTACGACGGGTTGGTAATG
 CCAGTGGCGTACCGTACCTATACTACTACTTGACCAAGGGATGCTGCCAACATTAC
 AlaGlnLeuLeuArgIleProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGly
 661 GCTCAGCTGCTCCGGATCCACAAGCCATCTGGACATGATCGCTGGCTCACTGGGA
 CGAGTCGACGAGGCCTAGGGTGTGGTAGAACCTGACTAGCGACCACGAGTGACCCCT
 ValLeuAlaGlyIleAlaTyrPheSerMetValGlyAsnTrpAlaLysValLeuValVal
 721 GTCCCTGGCGGGCATAGCGTATTCCTCCATGGTGGGAACTGGCGAAGGTCTGGTAGTG
 CAGGACCGCCCGTATCGCATAAAAGAGGGTACCAACCCCTTGACCCGCTCCAGGACCATCAC
 LeuLeuLeuPheAlaGlyValAspAlaGluThrHisValThrGlyGlySerAlaGlyHis
 781 CTGCTGCTATTGCGGGCGTCGACGCCAAACCCACGTCAACGGGGAAAGTGGCCGGCAC
 GACGACGATAAACGGCCGAGCTGCGCTTGGGTGAGTGGCCCCCTCACGGCCGGTG

FIGURE 16-1

841 Thr¹Al²Ser³Gly⁴Phe⁵Val⁶Ser⁷Leu⁸Leu⁹Ala¹⁰Pro¹¹Gly¹²Ala¹³Lys¹⁴Gln¹⁵Asn¹⁶Val¹⁷Gln¹⁸Leu¹⁹Ile²⁰
 ACTGIGTCTGGATTGTAGCCCTCCTCGACCAGGC²¹GC²²AAGCAGAACGTC²³CAGCTGATC²⁴TGACACAGACCTAAACAATCGGAGGAGCGTGGTCCCG²⁵GTCTTG²⁶CAGGT²⁷CGACTAG²⁸
 901 Asn¹Thr²Asn³Gly⁴Ser⁵Trp⁶His⁷Leu⁸Asn⁹Ser¹⁰Thr¹¹Ala¹²Leu¹³Asn¹⁴Cys¹⁵Asn¹⁶Asp¹⁷Ser¹⁸Leu¹⁹Asn²⁰
 AACACCAACGGCAGTTGGCACCTCAATAGCACGGCCCTGA²¹ACTGCAATGATAGC²²CTCAAC²³TTG²⁴TGGTTGCCGTCAACCGTGGAGT²⁵TATCG²⁶TGCCC²⁷GGACTTGACGT²⁸TACTATCGGAGTT²⁹
 961 Thr¹Gly²Trp³Leu⁴Ala⁵Gly⁶Leu⁷Phe⁸Fyr⁹His¹⁰His¹¹Lys¹²Phe¹³Asn¹⁴Ser¹⁵Ser¹⁶Gly¹⁷Cys¹⁸Pro¹⁹Glu²⁰
 ACCGGC²¹TGGTTGGCAGGGCTT²²TCTATCACCA²³ACAAGTTCAACTCTTCAGGCTG²⁴TGAG²⁵TGGCCGACCAACC²⁶GTCCC²⁷AAAAGATAGTGGT²⁸TCAAGTTGAGAAGTCCGACAGGACTC²⁹
 1021 Arg¹Leu²Ala³Ser⁴Cys⁵Arg⁶Pro⁷Leu⁸Thr⁹Asp¹⁰Phe¹¹Asp¹²Gly¹³Trp¹⁴Gly¹⁵Pro¹⁶Ile¹⁷Ser¹⁸Tyr¹⁹
 AGGCTAGCCAGCTGCCACCCCTTACCGATT²⁰TGACCAGGGCTGGGGCC²¹TATCAGTTAT²²TCCGATCGGTCGACGGCTGGGGAA²³TGGCTAA²⁴ACTGGTCCC²⁵GACCCGGGATAGTC²⁶ATAA²⁷
 1081 Ala¹Asn²Gly³Ser⁴Gly⁵Pro⁶Asp⁷Gln⁸Arg⁹Pro¹⁰Tyr¹¹Cys¹²Trp¹³His¹⁴Tyr¹⁵Pro¹⁶Pro¹⁷Lys¹⁸Pro¹⁹Cys²⁰
 GCCAACGGAAGCGGCCCGACCAGCGCCCTACTGCTGGCACTACCCCCAAACCTTGC²¹CGGTG²²CCCTCGCCGGGCTGGTCGCGGGGATGACGACC²³GTGATGGGGGTTTGGAACG²⁴
 1141 Gly¹Ile²Val³Pro⁴Ala⁵Lys⁶Ser⁷Val⁸Cys⁹Gly¹⁰Pro¹¹Val¹²Tyr¹³Cys¹⁴Phe¹⁵Thr¹⁶Pro¹⁷Ser¹⁸Pro¹⁹Val²⁰
 GGTATTGTGCCCGCGAAGAGTGTGTGGTCCGGTATATTGCTTC²¹ACTCCCAGCCCCGTG²²CCATAACACGGCCGCTTC²³CACACACCAGGCCATAAACGAAGT²⁴GAGGGTCGGGGC²⁵AC
 1201 Val¹Val²Gly³Thr⁴Thr⁵Asp⁶Arg⁷Ser⁸Gly⁹Ala¹⁰Pro¹¹Thr¹²Tyr¹³Ser¹⁴Trp¹⁵Gly¹⁶Glu¹⁷Asn¹⁸Asp¹⁹Thr²⁰
 GTGGTGGGAACGACGGACAGGT²¹CGGCCGCCCCACCTACAGCTGGGGT²²GAAAATGATA²³CGCACCCTTG²⁴TGCTGGTCCAGGCCGCGCCGGTGGATGTC²⁵GACCCACTTTACTATGC²⁶
 1261 Asp¹Val²Phe³Val⁴Leu⁵Asn⁶Asn⁷Thr⁸Arg⁹Pro¹⁰Pro¹¹Leu¹²Gly¹³Asn¹⁴Trp¹⁵Phe¹⁶Gly¹⁷Cys¹⁸Thr¹⁹Trp²⁰
 GACGTCTCGT²¹CTTAC²²AA²³TAC²⁴CGGCC²⁵ACCC²⁶CTGGCAATTGG²⁷TTCGG²⁸TG²⁹TACCTGG³⁰CTGCAGAACG³¹AGC³²GG³³ACATGGACC³⁴
 1321 Met¹Asn²Ser³Thr⁴Gly⁵Phe⁶Thr⁷Lys⁸Val⁹Cys¹⁰Gly¹¹Ala¹²Pro¹³Pro¹⁴Cys¹⁵Val¹⁶Ile¹⁷Gly¹⁸Gly¹⁹Ala²⁰
 ATGAACTCAACTGGATT²¹ACCAAA²²GTG²³CGGAGCGC²⁴CTCC²⁵TG²⁶TGTC²⁷ATCGGAGGGCG²⁸TACTTGAGTTGAC²⁹CTAAC³⁰CGC³¹CTG³²GG³³AG³⁴GG³⁵CG³⁶
 1381 Gly¹Asn²Asn³Thr⁴Leu⁵His⁶Cys⁷Pro⁸Thr⁹Asp¹⁰Cys¹¹Phe¹²Arg¹³Lys¹⁴His¹⁵Pro¹⁶Asp¹⁷Ala¹⁸Thr¹⁹Tyr²⁰
 GGCAACAAACACCC²¹TGCACTGCC²²CACTGAT²³TGCTCC²⁴GC²⁵AA²⁶GC²⁷AT²⁸CCGGACGCCACATAC²⁹CCGTTG³⁰TG³¹GGAC³²GT³³GA³⁴GG³⁵TG³⁶TATG³⁷
 1441 Ser¹Arg²Cys³Gly⁴Ser⁵Gly⁶Pro⁷Trp⁸Ile⁹Thr¹⁰Pro¹¹Arg¹²Cys¹³Leu¹⁴Val¹⁵Asp¹⁶Tyr¹⁷Pro¹⁸Tyr¹⁹Arg²⁰
 TCTCGGTGCGGCTCCG²¹GT²²CCCTGGAT²³CAC²⁴ACCC²⁵AGGT²⁶TG²⁷CTGGTC²⁸GA²⁹CT³⁰ACCCGT³¹TATAGG³²AGAGCC³³ACGCC³⁴GAGGCC³⁵AGGGAC³⁶CTAGT³⁷GTGGT³⁸CCACGGACCAGCTG³⁹ATGGGC⁴⁰ATATCC⁴¹
 1501 Leu¹Trp²His³Tyr⁴Pro⁵Cys⁶Thr⁷Ile⁸Asn⁹Tyr¹⁰Thr¹¹Ile¹²Phe¹³Lys¹⁴Ile¹⁵Arg¹⁶Met¹⁷Tyr¹⁸Val¹⁹Gly²⁰
 CTTGGCATT²¹TATCCTG²²TACCAT²³CAACT²⁴ACCCAT²⁵ATT²⁶AAA²⁷ATCAGGAT²⁸TGAC²⁹TG³⁰GG³¹A³²G³³T³⁴AG³⁵T³⁶GT³⁷AA³⁸TT³⁹TAG⁴⁰T⁴¹C⁴²CT⁴³AC⁴⁴AT⁴⁵GC⁴⁶ACCC⁴⁷
 1561 Gly¹Val²Glu³His⁴Arg⁵Leu⁶Glu⁷Ala⁸Ala⁹Cys¹⁰Asn¹¹Trp¹²Thr¹³Arg¹⁴Gly¹⁵Glu¹⁶Arg¹⁷Cys¹⁸Asp¹⁹Leu²⁰
 GGGGTCGAACACAGGC²¹TGGAAGCTGCC²²TGCA²³ACTGGACGCC²⁴GGCGAAC²⁵GTG²⁶CG²⁷AT²⁸CTGCC²⁹CCCCG³⁰CTTGCA³¹CGCTAGAC³²
 1621 Glu¹Asp²Arg³Asp⁴Arg⁵Ser⁶Glu⁷Leu⁸Ser⁹Pro¹⁰Leu¹¹Leu¹²Thr¹³Thr¹⁴Gln¹⁵Trp¹⁶Gln¹⁷Val¹⁸
 GAAGAC²¹AGGGAC²²AGGT²³CCGAG²⁴CT²⁵AG²⁶CCC²⁷GT²⁸TA²⁹CT³⁰G³¹AC³²AG³³T³⁴GG³⁵CA³⁶GT³⁷CG³⁸AG³⁹TC⁴⁰CC⁴¹AG⁴²GT⁴³AC⁴⁴CC⁴⁵GT⁴⁶CC⁴⁷AG⁴⁸AC⁴⁹CG⁵⁰AC⁵¹
 1681 Leu¹Pro²Cys³Ser⁴Phe⁵Thr⁶Thr⁷Leu⁸Pro⁹Ala¹⁰Leu¹¹Ser¹²Thr¹³Gly¹⁴Leu¹⁵Ile¹⁶His¹⁷Leu¹⁸His¹⁹Gln²⁰
 CTCCCGT²¹GTT²²CTT²³ACA²⁴ACC²⁵CT²⁶TACC²⁷AG²⁸GC²⁹CT³⁰GT³¹CC³²AC³³CC³⁴CT³⁵CC³⁶AC³⁷CC³⁸AG³⁹CG⁴⁰CC⁴¹AC⁴²GG⁴³TC⁴⁴
 1741 Asn¹Ile²Val³Asp⁴Val⁵Gln⁶Tyr⁷Leu⁸Tyr⁹Gly¹⁰Val¹¹Gly¹²Ser¹³Ile¹⁴Ala¹⁵Ser¹⁶Trp¹⁷Ala¹⁸Ile¹⁹
 AACATTGTGGACGTG²⁰CAGT²¹ACT²²TG²³AC²⁴GGG²⁵GT²⁶GGG²⁷TCA²⁸AG²⁹C³⁰AT³¹CGC³²GT³³CT³⁴GGCC³⁵ATT³⁶TTG³⁷TA³⁸AC³⁹CT⁴⁰GC⁴¹AC⁴²CG⁴³TAA⁴⁴
 1801 Lys¹Trp²Glu³Tyr⁴Val⁵Val⁶Leu⁷Leu⁸Phe⁹Leu¹⁰Leu¹¹Ala¹²Asp¹³Ala¹⁴Arg¹⁵Val¹⁶Cys¹⁷Ser¹⁸Cys¹⁹
 AAGTGGGAGTACGTG²⁰TTCT²¹GC²²TTG²³CT²⁴GC²⁵AG²⁶AC²⁷GC²⁸CG²⁹GT³⁰CTG³¹CT³²GC³³

FIGURE 16-2

1861 TTCACCCCTCATGCAGCAAGAGGACAAGGAAGACGAACGTCTGCGCGCAGACGAGGACG
 LeuTrpMetMetLeuLeuIleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeu
 TTGTGGATGATGCTACTCATATCCCCAACGGGAGGGCTTGGAGAACCTCGTAATACTT
 AACACCTACTACGATGAGTATAGGGTCCCTCCGCCAACCTCTGGAGCATTATGAA
 AsnAlaAlaSerLeuAlaGlyThrHisGlyLeuValSerPheLeuValPhePheCysPhe
 1921 AATGCAGCATCCCTGGCCGGACGGCACGGTCTTGTATCCTTCGTGTCTCTGCTTT
 TTACGTCTAGGGACCAGGCCCTGCGTGCCAGAACATAGGAAGGAGCACNAGAAGACGAAA
 AlaTrpTyrLeuLysGlyLysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrp
 1981 GCATGGTATTGAAGGGTAAGTGGGTGCCCGAGCGGTCTACACCTTACGGGATGTGG
 CGTACCATAAACTTCCCATTCAACCCACGGGCCCTGCCAGATGTGAAGATGCCCTACACC
 ProLeuLeuLeuLeuLeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluVal
 2041 CCTCTCCTCTGCTCTGTTGGCGTTGCCCGACGGGCGTACCGCTGGACACGGAGGGTG
 GGAGAGGAGGAGCAGAGCAACCGAACGGGTGCCATGCCGACCTGTGCCCTCAC
 AlaAlaSerCysGlyGlyValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyr
 2101 GCCGCCTCGTGTGGCGGTGTTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATAT
 CGCGCAGCACACCGCCAACAAGACGAGCCAACTACCGGACTGAGACAGTGGTATA
 TyrLysArgTyrIleSerTrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGlu
 2161 TACAAGCGCTATATCAGCTGGTCTGCTGGCTTCAGTATTTCTGACCAAGACTGGAA
 ATGTTCGCGATATAGTCGACCACGAAACCACCGAACAGTCATAAAAGACTGGTCTCACCTT
 AlaGlnLeuHisValTrpIleProProLeuAsnValArgGlyGlyArgAspAlaValIle
 2221 GCGCAACTGACGTGTGGATTCCCCCTCAACGTCGAGGGGGCGCGACGCCGTCACTC
 CGCGTTGACGTGACACCTAACGGGAGTTGAGCCTGCCGCTGCCGCTGCCAGTAG
 LeuLeuMetCysAlaValHisProThrLeuValPheAspIleThrLysLeuLeuLeuAla
 2281 TTACTCATGTGCTGTACACCCGACTCTGGTATTGACATCACCAAATTGCTGCTGGCC
 AATGAGTACACACGACATGGGCTGAGACCATAACTGTAGTGGTTAACGACGACCGG
 ValPheGlyProLeuTrpIleLeuGlnAlaSerLeuLeuLysValProTyrPheValArg
 2341 GTCTTCGGACCCCTTGGATTCTCAAGCCAGTTGCTTAAAGTACCCCTACTTGTCGCG
 CAGAACGCTGGGAAAACCTAACGAAAGTCTGGTCAAACGAATTTCATGGGATGAAACACGCG
 ValGlnGlyLeuLeuArgPheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrVal
 2401 GTCCAAGGCCCTCTCGGGTTCTGCGCTTAGCGCGGAAGATGATCGGAGGCCATTACGTG
 CAGGTTCCGGAAAGAGGCCAACGCGCAATCGCCCTCTACTAGCCTCCGTAATGCA
 GlnMetValIleIleLysLeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThr
 2461 CAAATGGTCATCTTAAGTTAGGGCGCTTACTGGCACCTATGTTATAACCATCTCACT
 GTTACCACTAGTAATTCAATCCCCGCAATGACCGTGGATACAAATATGGTAGGTGA
 ProLeuArgAspTrpAlaHisAsnGlyLeuArgAspLeuAlaValAlaValGluProVal
 2521 CCTCTCGGGACTGGCGCACACGGCTGCGAGATCTGGCGTGGCTGTAGAGCCAGTC
 GGAGAACCCCTGACCCCGCTGTTGCCAACGCTTAGACCGGCCACCGACATCTGGTCAG
 ValPheSerGlnMetGluThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGly
 2581 GTCTTCTCCAAATGGAGACCAAGCTCATCACGTGGGGGGCAGATACGCCCGTGCCT
 CAGAACAGGGTTACCTCTGGTTCGAGTAGTGCACCCCCCGTCTATGGCGGCCACGCCA
 AspIleIleAsnGlyLeuProValSerAlaArgArgGlyArgGluIleLeuLeuGlyPro
 2641 GACATCATCACGGCTTGCCTTTCCGCCCGCAGGGCGGGAGATACTGCTCGGGCCA
 CTGTAGTAGTGGCGAACGGACAAAGCGGGCGTCCCCGGCCCTCTATGACGAGCCGGT
 AlaAspGlyMetValSerLysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGln
 2701 GCGGATGGAATGGTCTCCAAGGGTGGAGGTGCTGGCCCCATCACGGCTACGCCAG
 CGGCTACCTTACCAAGAGGTTCCCACCTCAACGACCGCGGGTAGTGCCTGAC
 GlnThrArgGlyLeuLeuGlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGln
 2761 CAGACAAAGGGGCCCTAGGGTGCATAATCACCAGCCTAACTGGCCGGGACAAAAACCAA
 GTCTGTTCCCCGGAGGATCCCACGTATTAGTGGTGGATTGACCGGCCCTGTTTGGTT

FIGURE 16-3

2821 ValGluGlyGluValGlnIleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIle
 GIGGAGGGTGAGGTCCAGATTGTCAACTGCTGCCAACCTTCTGGCAACGTGCATC
 CACCTCCCACCTCCAGGTCTAACACAGTTGACGACGGGTTGGAAGGACCGTTGACGTAG

 2881 AsnGlyValCysTrpThrValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLys
 AATGGGGTGTGCTGACTGTCTACCACGGGCCGGAACAGGACCATCGGTCAACCAAG
 TTACCCCCACACGACCTGACAGATGGTGCCCCGGCTTGCTCTGGTAGCGCAGTGGGTT

 2941 GlyProValIleGlnMetTyrThrAsnValAspGlnAspLeuValGlyTrpProAlaPro
 GGTCTGTCACTCCAGATGTATAACCAATGTAGACCAAGACCTTGTGGCTGGCCCGCTCCG
 CCAGGACAGTAGGTCTACATAGGTACATCTGGTCTGGAACACCCGACCGGGCGAGGC

 3001 GlnGlySerArgSerLeuThrProCysThrCysGlySerSerAspLeuTyrLeuValThr
 CAAGGTAGCCGCTCATGGACACCCCTGCACTTGCGGCTCTGGACCTTACCTGGTCACG
 GTTCCATGGCGAGTAACGTGGACGTGAAAGCCGAGGAGCCTGGAATGGACAGTGC

 3061 ArgHisAlaAspValIleProValArgArgArgGlyAspSerArgGlySerLeuLeuSer
 AGGCACGCCGATGTCATTCCCGTGCACGGCGGGGTGATAGCAGGGCAGCCTGTCAG
 TCCGTGCGGCTACAGTAAGGGCACGCCGCCCCACTATCGTOCCCCTGGACGACAGC

 3121 ProArgProIleSerTyrLeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGly
 CCCCCGGCCCATTCCCTACTTGAAAGGCTCTCGGGGGTCCGCTGTGTCAGGGCGGGGG
 GGGGGCGGGTAAAGGATGAACCTTCCGAGGAGCCCCCAGCGACACAGGGCGCCCC

 3181 HisAlaValGlyIlePheArgAlaAlaValCysThrArgGlyValAlaLysAlaValAsp
 CACGCCGTGGGCATTTAGGGCCGGGTGTCACCCGTTGGAGTGGCTAAGGCCGGTGGAC
 GTGCGGCACCCGTATAAATCCGGGCCACACGTGGGACCTCACCGATTCCGCCACCTG

 3241 PheIleProValGluAsnLeuGluThrThrMetArgSerProValPheThrAspAsnSer
 TTTATCCCTGTGGAGAACCTAGAGACAACCATGAGGTCCCCGGTGTTCACGGATAACTCC
 AAATAGGGACACCTCTGGATCTCTGGTACTCCAGGGCCACAAGTGCCTATTGAGG

 3301 SerProProValValProGlnSerPheGlnValAlaHisLeuHisAlaProThrGlySer
 TCTCCACCACTAGTGGCCAGAGCTTCAGGTGGCTCACCTCCATGCTCCACAGGCAGC
 AGAGGTGGTCATCACGGGTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTG

 3361 GlyLysSerThrLysValProAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeu
 GGCAAAAGCACCAAGTCCGGCTGCATATGCAGCTCAGGGCTATAAGGTGCTAGTACTC
 CCGTTTCGIGGTTCCAGGGCGACGTACGTCAGTCCCATACTCCACGATCATGAG

 3421 AsnProSerValAlaAlaAlaThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIle
 AACCCCTCTGGCTGCAACACTGGGCTTGCTTACATGTCAAGGCTCATGGATC
 TTGGGGAGACAACGACGGTGTGACCCGAAACCAACGAATGTACAGGTCCGAGTACCC

 3481 AspProAsnIleArgThrGlyValArgThrIleThrThrGlySerProIleThrTyrSer
 GATCCTAACATCAGGACCGGGGTGAGAACAAATTACCACTGGCAGCCCCATCACGTACTCC
 CTAGGATGTAGTCTGGCCCCACTCTGTTAATGGTGACCGTCGGGGTAGTGCATGAGG

 3541 ThrTyrGlyLysPheLeuAlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIle
 ACCTACGGCAAGTCTCTGGCAGGGGGTGCTCGGGGGCGTTATGACATAATAATT
 TGGATGCCGTTCAAGGAACGGCTGCCACGAGCCCCCGCGAATACTGTATTATTAA

 3601 CysAspGluCysHisSerThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAsp
 TGTGACGAGTGCCACTCCACGGATGCCACATCCATCTGGCAGTGGCACTGTCCTTGAC
 AACATGCTCACGGTGAGGTGCTACGGTGTAGGTAGAACCCGTACCGGTACAGGAAC

 3661 GlnAlaGluThrAlaGlyAlaArgLeuValValLeuAlaThrAlaThrProProGlySer
 CAAGCAGAGACTGCCGGGGCGAGACTGGTTGCTCGCCACCGCCACCCCTCCGGCTCC
 GTTCGTCTCTGACGCCCGCTCTGACCAACAGAGCGGTGGCGGTGGGGAGGCCGAGG

 3721 ValThrValProHisProAsnIleGluGluValAlaLeuSerThrThrGlyGluIlePro
 GTCACTGTGCCCCATCCCAACATCGAGGAGGTGCTCTGTCACCACCGAGAGATCCCT
 CAGTGACACGGGTAGGGTTGAGCTCCTCAACGAGACAGGTGGTGGCTCTAGGGA

 3781 PheTyrGlyLysAlaIleProLeuGluValIleLysGlyGlyArgHisLeuIlePheCys
 TTTTACGGCAAGGCTATCCCCCTGAAAGTAATCAAGGGGGGAGACATCTCATCTCTGT

FIGURE 16-4

AAAATGCCGTTCCGATAGGGGGAGCTTCATTAGTTCCCCCTCTGTAGAGTAGAAGACA
 3841 HisSerLysLysLysCysAspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAla
 CATTCAAAGAAGAAGTGCACGAAACTCGCCGAAAGCTGGTCGCATTGGCATCAATGCC
 GTAAAGTTCTCTTCACGCTGCTGAGCGGGTTTCGACCAGCATAACCGTAGTTACGG
 3901 ValAlaTyrTyrArgGlyLeuAspValSerValIleProThrSerGlyAspValValVal
 GTGGCCTACTACCGCGGCTTGACGTGTCCGTACATCCGACAGCGCGATGTTGTCGTC
 CACCGGATGATGGCGCCAGAACCTGCACAGCAGTAGGGCTGGTCGCCCTACAACAGCAG
 3961 ValAlaThrAspAlaLeuMetThrGlyTyrThrGlyAspPheAspSerValIleAspCys
 GTGGCAACCGATGCCCTATGACCGCTATACCGCGACTTCGACTCGGTGATAGACTGC
 CACCGTTGGCTACGGGAGTAECTGGCGATATGGCGCTGAAGCTGAGCCACTATCTGACG
 4021 AsnThrCysValThrGlnThrValAspPheSerLeuAspProThrPheThrIleGlutThr
 AATACGTGTGTCACCCAGACAGTCGATTTCAGCCTGACCCCTACCTCACCCATTGAGACA
 TTATGCACACAGTGGGTCTGTCAGCTAAAGTCGAACTGGGATGGAAGTGGTAACCTGT
 4081 IleThrLeuProGlnAspAlaValSerArgThrGlnArgArgGlyArgThrGlyArgGly
 ATCACGCTCCCCCAGGAATGCTGTCCTCCGCACCTCACAGTCGGGAGGACTGGCAGGGGG
 TAGTGGCAGGGGGTCTACGACAGAGGGCGTGAGTTGAGCCCCCTGCGGGGAGGCCGTACAAGCTGAGC
 4141 LysProGlyIleTyrArgPheValAlaProGlyGluArgProSerGlyMetPheAspSer
 AAGCCAGGCATCTACAGATTGTCGGCACCGGGGAGCGCCCTCCGGCATGTTGACTCG
 TTGGTCCGTAGATGTCATAACACCGTGGCCCCCTCGCGGGAGGCCGTACAAGCTGAGC
 4201 SerValLeuCysGluCysTyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGlu
 TCCGTCTCTGTGAGTGCTATGACCGAGGCTGTGCTTGTATGAGCTCACGCCGCCAG
 AGGCAGGAGACACTCACGATACTGCGTCCGACACGAAACCATACTCGAGTGGGGCGGCTC
 ThrThrValArgLeuArgAlaTyrMetAsnThrProGlyLeuProValCysGlnAspHis
 4261 ACTACAGTTAGCTACGAGCGTACATGAAACACCCGGGGCTTCCGTGTGCCAGGACCAT
 TGATGTCATCCGATGCTCGCATGTAATTGAGGGGGGGGGGGGGGGGGGGGGGGGGGGGG
 4321 LeuGluPheTrpGluGlyValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSer
 CTTGAATTGGGGAGGGCGTCTTACAGGCTCACTCATATAGATGCCACTTCTATCC
 GAACTTAAACCTCCCGCAGAAATGTCGGAGTGAGTATATCACGGGTGAAAGATAGG
 4381 GlnThrLysGlnSerGlyGluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCys
 CAGACAAAGCAGAGTGGGGAGAACCTCTTACCTGGTAGCGTACCAAGCCACCGTGTGC
 GTCTGTTCTGTCACCCCTCTGGAGGAATGGACCATCGCATGGTTCGGTGGCACACCG
 4441 AlaArgAlaGlnAlaProProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeu
 GCTAGGGCTCAAGCCCCCTCCCATCGTGGGACCAGATGAGTGGAAAGTGGTATTGCGCTC
 CGATCCGAGTTCGGGGAGGGGGTAGCACCTGGTCTACACCTTCACAAACTAACGGGAG
 4501 LysProThrLeuHisGlyProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGlu
 AAGCCCACCCCTCATGGGCCAACACCCCTGCTATAACAGACTGGGCGTGTCAAGATGAA
 TTCGGGTGGGAGGTACCCGGTTGAGGGACGATATGTCGACCCGCGACAAGCTTACTT
 4561 IleThrLeuThrHisProValThrLysTyrIleMetThrCysMetSerAlaAspLeuGlu
 ATCACCCCTGACGCACCCAGTCACCAAATACATCATGACATGCGATGCGGCCGACCTGGAG
 TAGTGGGACTGCGTGGGTAGTGGTTATGTAGTACTGTACGTACAGCCGGCTGGACCTC
 4621 ValValThrSerThrTrpValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCys
 GTCGTACGAGCACCTGGGTGCTCGTGGCGCGTCTGGCTGCTTGGCCCGTATTGCG
 CAGCAGTGTGTCGACGAGCAGCAACGCCAGGACGGACCGACGAAACCGGGCGATAACG
 4681 LeuSerThrGlyCysValValIleValGlyArgValLeuSerGlyLysProAlaIle
 CTGTCAACAGGCTGCGTGGTCATAGTGGGCAGGGTGTCTGTCGGGAAGCCGGCAATC
 GACAGTTGTCGACGCACCGAGTATCACCGTCCCAGCAGAACAGGCCCTCGGGCGTTAG
 4741 IleProAspArgGluValLeuTyrArgGluPheAspGluMetGluGluCysSerGlnHis
 ATACCTGACAGGGAAAGTCCTCTACCGAGAGTTCGATGACATGGAAGAGTGCTCTCAGCAC
 TATGGACTGTCCTTCAGGAGATGGCTCTCAAGCTACTCAGCTACGAGAGTCGTG

FIGURE 16-5

4801 Le_nProTyrlleGluGlnGlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGly
 TTACCGTACATCGAGCAAGGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGC
 AATGGCATGTAGCTCGTCCCCACTACGAGCGCTCGTCAGTTGCTTCCGGGAGCCG
 4861 LeuLeuGlnThrAlaSerArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrp
 CTCCCTGGAGACCGCGTCCCGTCAAGGAGGTATGCCCTGCTGTCAGACCAACTGG
 GAGGACGTCTGGCGCAGGGCAGTCGGTCCAATAGCGGGGACGACAGGTCTGGTTGACC
 4921 GlnLysLeuGluThrPheTrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyr
 CAAAAACTCGAGACCTTCGGCGAACATATGCGAACTTCATCAGTGGGATAACAATAC
 GTTTTGAGCTCTGGAAGACCCGCTTCGTATACACCTGAAAGTAGTAGTCACCCATGTTATG
 4981 LeuAlaGlyLeuSerThrLeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThr
 TTGGCGGGCTTGTCAACGCTGGTAACCCCGCCATTGCTTCATTGATGGCTTTTACA
 AACCGCCCGAACAGTTGCGACGGACATTGGGGCGTAACGGAGTAACTACCGAAAATGT
 5041 AlaAlaValThrSerProLeuThrSerGlnThrLeuLeuPheAsnIleLeuGlyGly
 GCTGCTGTCAACCAGCCCCTAACCAACTAGCCAAACCCCTCTTCACATATGGGGGG
 CGACGACAGTGGTGGGTGATTGGTGATCGGTTGGGAGGAGAAGTTGTATAACCCCCCCC
 5101 TrpValAlaAlaGlnLeuAlaAlaProGlyAlaAlaThrAlaPheValGlyAlaGlyLeu
 TGGGTGGCTGCCAGCTCGCCGGGGTGCCGCTACTGCTTTGTCGGCGCTGGCTTA
 ACCCACCGACGGGTGAGCGGGGGGCCACGGCGATGACGGAAACACCCGCGACCGAAT
 5161 AlaGlyAlaAlaIleGlySerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGly
 GCTGGCGCCGCCATCGGCAGTGGACTGGGAAGGTCTCATAGACATCCTGCAGGG
 CGACCGCGCGGTAGCCGTACAACCTGACCCCTCCAGGAGTATCTGTAGGAACGTCCC
 5221 TyrGlyAlaGlyValAlaGlyAlaLeuValAlaPheLysIleMetSerGlyGluValPro
 TATGGCGGGCGTGGCGGGAGCTCTTGTCATTGCAAGATCATGAGCGGTGAGGTCCCC
 ATACCGGCCCGCACCGCCCTCGAGAACACCGTAAGTCTAGTACTCGCCACTCAGGGG
 5281 SerThrGluAspLeuValAsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValVal
 TCCACGGAGGACCTGGTCAATCTACTGCCCCCCTCGCCGGAGCCCTCGTAGTC
 AGGTGCCCCCTGGACCAGTTAGATGACGGCGGTAGGAGAGCGGGCTCGGGAGCAG
 5342 GlyValValCysAlaAlaIleLeuArgArgHisValGlyProGlyGluGlyAlaValGln
 GGCCTGGCTGTGCAGCAATACTGCGCCGGACGTGGCCGGAGGGGGAGTGCAG
 CGCGACACAGACACGTCGTTATGACCGCCGTGCAACCGGGCCGCTCCCCCGTCACGTC
 5401 TrpMetAsnArgLeuIleAlaPheAlaSerArgGlyAsnHisValSerProThrHisTyr
 TGGATGAACCGGCTGATAGCCTCGCCTCCGGGGAACCATGTTCCCCCACGCACTAC
 ACCTACTTGGCCGACTATCGGAAGCGGAGGGCCCCCTGGTACAAAGGGGTGCGTGATG
 5461 ValProGluSerAspAlaAlaAlaArgValThrAlaIleLeuSerSerLeuThrValThr
 GTGCCGGAGAGCGATGCAGCTGCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACC
 CACGGCCCTCGCTACGTCGACGGCGCAGTACGGTATGAGTCGGAGTGAACATTGG
 5521 GlnLeuLeuArgArgLeuHisGlnTrpIleSerSerGluCysThrThrProCysSerGly
 CAGCTCTGAGGGCAGTCACCACTGGATAAGCTGGAGTGTACCACTCCATGCTCCGGT
 GTCGAGGACTCCGCTGACGTGGTACCTATTGAGCCTCACATGGTAGGTACGAGGGCCA
 5581 SerTrpLeuArgAspIleTrpAspTrpIleCysGluValLeuSerAspPheLysThrTrp
 TCCTGGCTAAGGGACATCTGGACTGGATAAGCTGGAGTGTACCACTTAAAGACCTGG
 AGGACCGATTCCCTGTAGACCCCTGACCTATAAGCTCCACATGGTAAATTCTGGACC
 5641 LeuLysAlaLysLeuMetProGlnLeuProGlyIleProPheValSerCysGlnArgGly
 CTAAAAGCTAAGCTCATGCCACAGCTGCCCTGGATCCCCCTTGTCCTGCCAGCGCGGG
 GATTTTCGATTGAGTACGGTGTGACGGACCCCTAGGGGAAACACAGGACGGTCGCGCCC
 5701 TyrLysGlyValTrpArgValAspGlyIleMetHisThrArgCysHisCysGlyAlaGlu
 TATAAGGGGGCTGGCGAGTGGACGGCATATGCAACTCGCTGCCACTGTGGAGCTGAG
 ATATTCCCCCAGACCGCTCACCTGGTAGTACGTGTGAGCGACGGTGACACCTCGACTC
 5761 IleThrGlyHisValLysAsnGlyThrMetArgIleValGlyProArgThrCysArgAsn
 ATCACTGGACATGTCAAAAACGGGACGATGAGGATGTCGGTCCTAGGACCTGCAGGAAC

FIGURE 16-6

TAG TGACCTGTACAGTTTGCCTGCTACTCCTAGCAGCCAGGATCTGGACGTCCCTG
 MetTrpSerGlyThrPheProIleAsnAlaTyrThrThrGlyProCysThrProLeuPro
 5821 ATGGGAGTGGGACCTCCCCATTAAATGCCCTACACCACGGGCCCTGTACCCCCCTTCCT
 TACACCTCACCCCTGGAAGGGTAATTACGGATGTGGTGCCCGGGACATGGGGGGAGGA
 AlaProAsnTyrThrPheAlaLeuTrpArgValSerAlaGluGluTyrValGluIleArg
 5881 GCGCCGAACTACACGTCGCGCTATGGAGGGTGTCTGCAGAGGAATATGGAGATAAGG
 CGCGGCTTGATGTGCAAGCGCGATACCTCCCACAGACGTCTCCTTATACACCTCTATTCC
 GlnValGlyAspPheHisTyrValThrGlyMetThrThrAspAsnLeuLysCysProCys
 5941 CAGGTGGGGGACTTCCACTACGTGACGGGTATGACTACTGACAATCTCAAATGCCGTGC
 GTCCACCCCTGAAGGTGATGCACTGCCACTGTGACTGTTAGAGTTACGGGCACG
 GlnValProSerProGluPhePheThrGluLeuAspGlyValArgLeuHisArgPheAla
 6001 CAGGTCCCATGCCCGAATTTTACAGAACGGTGTGCGCTACATAGGTTGCG
 GTCCAGGGTAGCGGGCTTAAAGTGTCTAACCTGCCACGGGATGTATCCAACACGC
 ProProCysLysProLeuLeuArgGluGluValSerPheArgValGlyLeuHisGluTyr
 6061 CCCCCCTGCAAGCCCTGCTGCGGGAGGAGGTATCAGAGTAGGACTCCACGAATAC
 GGGGGGACGTTGGGAAACGACGCCCTCCATAGTAAGTCTCATCTGAGGTGCTTATG
 ProValGlySerGlnLeuProCysGluProGluProAspValAlaValLeuThrSerMet
 6121 CCGGTAGGGTCGCAATTACCTTGCAGGCCGAACCGACGTTGGCGTGTGACGTCATG
 GCCATCCCAGCGTTAATGGAACGCTCGGGCTTGGCTGCACCGGACAACACTGCAAGGTAC
 LeuThrAspProSerHisIleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySer
 6181 CTCACTGATCCCCTCCCATATAACAGCAGAGGCCGGCGAAGGTTGGCGAGGGGATCA
 GAGTGACTAGGGAGGGTATATGTGCTCTCCGGCCGGCTTCCAACCGCTCCCTAGT
 ProProSerValAlaSerSerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThr
 6241 CCCCCCTCTGGCCAGCTCCTCGGCTAGCCAGCTATCCGCTCCATCTCAAGGCAACT
 GGGGGGAGACACCGGTGAGGAGCCGATCGGTGCGTAGGCGAGGTAGAGAGTTCCGTG
 CysThrAlaAsnHisAspSerProAspAlaGluIleGluAlaAsnLeuLeuTrpArg
 6301 TGCACCGCTAACCATGACTCCCTGATGCTGAGCTCATAGAGGCCAACCTCTATGGAGG
 ACGTGGCGATTGGTACTGAGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCC
 GlnGluMetGlyGlyAsnIleThrArgValGluSerGluAsnLysValValIleLeuAsp
 6361 CAGGAGATGGCGGCAACATCACCAAGGGTGAGTCAGAAAACAAAGTGGTGAATTCTGGAC
 GTCTCTACCCGCCGTTGTAGTGGTCCCAACTCAGTCTTGTACCAACTAACGACCTG
 SerPheAspProLeuValAlaGluGluAspGluArgGluIleSerValProAlaGluIle
 6421 TCCTTCGATCCGCTTGCGAGGAGGACGAGCGGGAGATCTCGTACCCGAGAAATC
 AGGAAGCTAGCGAACACCGCTCCCTCGTACCCGAGTACGGCATGGCGTCTTGT
 LeuArgLysSerArgArgPheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsn
 6481 CTGCGGAAGTCTCGGAGATTGCGCCAGGCCCTGCCGTTGGCGCGCGGACTATAAC
 GACGCCCTCAGAGCCTCTAACGGGTCGGGACGGCAAAACCGCGCCGCTGATATTG
 ProProLeuValGluThrTrpLysProAspTyrGluProProValValHisGlyCys
 6541 CCCCCGCTAGTGGAGACGTGGAAAAGCCGACTACGAACCACTGTGGTCCATGGCTGT
 GGGGGCGATACCTCTGACCTTTGGGCTGATGCTGGTGGACACCAAGGTACCGACA
 ProLeuProProProLysSerProProValProProProArgLysLysArgThrValVal
 6601 CCGCTTCCACCTCCAAAGTCCCCTCTGCGCTCCGCTCGAAGAAGCGGACGGTGGC
 GCGAAGGTGGAGGTTCAAGGGAGGACACGGAGGCGGAGCCTCTCGCCACCAG
 LeuThrGluSerThrLeuSerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySer
 6661 CTCACTGAATCAACCTATCTACTGCCCTGGCGAGCTCGCACCAGAACGCTTGGCAGC
 GAGTGACTTAGTGGGATAGATGACCGAACCGGCTCGAGCGGIGGTCTCGAAACCGTCG
 SerSerThrSerGlyIleThrGlyAspAsnThrThrSerSerGluProAlaProSer
 6721 TCCTCAACTCCGGCATACGGCGACAATACGACAACATCCTCTGAGCCCGCCCTCT
 AGGAGTTGAAGGCGCTAATGCCGCTGTTATGCTGTTAGGAGACTCGGGCGGGGAAGA

6781 GlyCysProProAspSerAspAspAlaGluSerTyrSerSerMetProProLeuGluGlyGlu
 GGCCTGCCCCCCCGACTCCGACGCTGAGTCCTATTCCCATGCCCTGGAGGGGGAG
 CCGACGGGGGGGCTGAGGCTGCAGTCAGGATAAGGAGGTACGGGGGGACCTCCCCCTC
 6841 ProGlyAspProAspLeuSerAspGlySerTrpSerThrValSerSerGluAlaAsnAla
 CCTGGGGATCCGGATCTTAGCGACGGGTCAAGGTCAAGGTCAAGTGAGGCAACGCG
 GGACCCCTAGGCCAGAATCGCTGCCAGTACCACTGCCCAGTCATCACTCCGGTTGCGC
 6901 GluAspValValCysCysSerMetSerTyrSerTrpThrGlyAlaLeuValThrProCys
 GAGGATGTCGTGCTGCTCAATGTCTTACTCTGGACAGGCGCACTCGTCACCCGTGC
 CTCCCTACAGCACACGACGGAGTTACAGAATGAGAACCTGTCCCGTGAGCAGTGGGGACG
 6961 AlaAlaGluGluGlnLysLeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHis
 GCCGCAGAAACAGAAACTGCCCATCAATGCACTAACGAACTCGTGTACGTACCCAC
 CGCGCCTCTTGCTTTGACGGGTAGTTACGTGATTGAGCACGATGCACTGGTG
 7021 AsnLeuValTyrSerThrThrSerArgSerAlaCysGlnArgGlnLysLysValThrPhe
 AATTGGGTATTCCACCACCTCACCGCAGTGTGCAAAGGACAGAAAGTCACATTT
 TTAAACACATAAGGTGGTGGAGTGGCTCACGAACGGTTCCGTCTTCAGTGTAAA
 7081 AspArgLeuGlnValLeuAspSerHisTyrGlnAspValLeuLysGluValLysAlaAla
 GACAGACTGCAAGTTCTGGACAGCCATTACCAAGGACGTACTCAAGGAGGTTAACGAGCG
 CTGTCTGACGTTCAAGACCTGTGGTAATGGCTCTGCATGAGTTCCCTCAATTTCGTGCG
 7141 AlaSerLysValLysAlaAsnLeuLeuSerValGluGluAlaCysSerLeuThrProPro
 GCGTCAAAAGTGAAGGCTAACCTGCTATCCGTAGAGGAAGCTTGAGCCTGACGCCCA
 CGCAGTTTCACTCCGATTGAACGATAGGCATCTCTCGAACGTCGGACTGCGGGGT
 7201 HisSerAlaLysSerLysPheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLys
 CACTCAGCCAATCCAAGTTGGTATGGGGCAAAAGACGTCCGTGCCATGCCAGAAAG
 GTGAGTCGGTTAGGTCAAACCAATACCCGTTCTGCAGGCAACGGTACGGTCTTC
 7261 AlaValThrHisIleAsnSerValTrpLysAspLeuLeuGluAspAsnValThrProIle
 GCCGTAAGCCACATCAACTCCGTGGAAAGACCTCTGGAAAGACATGTAACACCAATA
 CGGCATTGGGTGTAGTTGAGGCACACCTTCTGGAAGACCTCTGTTACATTGTGGTTAT
 7322 AspThrThrIleAsnAlaLysAsnGluValPheGlyValGlnProGluLysGlyGlyArg
 GACACTACCACATGCTAACGAGGTTCTGGCTCGCCTGAGAACGGGTGCG
 CTGTGATGGTAGTACCGATTCTGCTCCAAAAGACGCAAGTCGGACTCTCCCCCAGCA
 7381 LysProAlaArgLeuIleValPheProAspLeuGlyValArgValCysGluLysMetAla
 AAGCCAGCTCGTCATCGTGTGTTCCCGATCTGGCGTGCCTGCGAAAGATGGCT
 TTCGGTCAGCAGAGTAGCACAAGGGGCTAGACCCGCACCGCACACGCTTCTACCGA
 7441 LeuTyrAspValValThrLysLeuProLeuAlaValMetGlySerSerTyrGlyPheGln
 TTGTACGACGTGGTTACAAAGCTCCCTGGCGTGATGGGAAGCTCCTACGGATTCAA
 AACATGCTGCACCAATGTTGAGGGGAAACCCGCACTACCCCTCGAGGATGCCATAAGGTT
 7501 TyrSerProGlyGlnArgValGluPheLeuValGlnAlaTrpLysSerLysLysThrPro
 TACTCACAGGACAGCGGGTGAATTCTCGTCAAGCGTGGAAAGTCCAAGAAAAACCCCA
 ATGAGTGGCTCTGCGCCCAACTTAAGGAGCACGTTGCACCTCAGGTTCTGGGGT
 7561 MetGlyPheSerTyrAspThrArgCysPheAspSerThrValThrGluSerAspIleArg
 ATGGGGTCTCGTATGATAACCCGCTGCTTGACTCCACAGTCACTGAGAGCGACATCCG
 TACCCCAAGAGCATACTATGGCGACGAAACTGAGGTGTCAGTGA
 7621 ThrGluGluAlaIleTyrGlnCysCysAspLeuAspProGlnAlaArgValAlaIleLys
 ACGGAGGAGGCAATCTACCAATGTTGACCTCGACCCCCAAGCCCCGGCTGCCATCAAG
 TGCCCTCCCGTTAGATGGTACAAACACTGGAGCTGGGGTTCGGGCGACCGGTAGTTC
 7681 SerLeuThrGluArgLeuTyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCys
 TCCCTCACCGAGAGGCTTATGTTGGGGCCCTCTTACCAATTCAAGGGGGAGAACTGC
 AGGGAGTGGCTCTCCGAAATACAACCCCGGGAGAATGGTAAAGTCCCCCTCTTGACG
 7741 GlyTyrArgArgCysArgAlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThr
 GGCTATCGCAGGTGCCCGCGAGCGCGTACTGACAACTAGCTGTGTAACACCCCTCACT

CCGATAGCGTCCACGGCGCTGCCGCATGACTGTTGATCGACACCATTGTGGGAGTGA
 CysTyrIleLysAlaArgAlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeu
 .7801 TGCTACATCAAGGCCGGGCAGCCTGTCGAGCCGCAGGGCTCCAGGACTGACCATGCTC
 ACGATGTAGTTCCGGGCCCCGTCGGACAGCTCGCGTCCCAGGTCTGACGTGGTACGAG
 ValCysGlyAspAspLeuValIleCysGluSerAlaGlyValGlnGluAspAlaAla
 7861 GTGTGTGGCGACGACTTAGTCGTTATCTGTAAAGCGCGGGGTCCAGGAGGACGCCCG
 CACACACCGCTGCTGAATCAGCAATAGACACTTTCGCGCCCCCAGGTCTCCTGCGCCGC
 SerLeuArgAlaPheThrGluAlaMetThrArgTyrSerAlaProProGlyAspProPro
 7921 AGCCTGAGAGCCTTCACGGAGGCTATGACCAGGTACTCCGCCCTGGGACCCCCCA
 TCGGACTCTCGGAAGTGCCTCCGATACTGGTCATGAGGGGGGGGACCCCTGGGGGT
 GlnProGluTyrAspLeuGluLeuIleThrSerCysSerSerAsnValSerValAlaHis
 7981 CAACCAGAACATCGACTTGGAGCTATAACATCAIGCTCCTCAACGTGTCAGTCGCCAC
 GTTGGTCTTATGCTGAACCTCGAGTATTGTAGTACGAGGGTTGCACAGTCAGCGGGTG
 AspGlyAlaGlyLysArgValTyrTyrLeuThrArgAspProThrThrProLeuAlaArg
 8041 GACGGCGCTGGAAAGAGGGTCTACTACCTCACCCGTGACCTACAACCCCCCTCGCGAGA
 CTGCCCGCACCTTCTCCAGATGATGGAGTGGGACTGGATGTTGGGGAGCGCTCT
 AlaAlaTrpGluThrAlaArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMet
 8101 GCTGCGTGGGAGACAGCAAGACACACTCCAGTCATTCTGGCTAGGAAACATAATCATG
 CGACGCACCCCTGTCGTTCTGTGAGGTCAAGGACCGATCCGTGTATTAGTAC
 PheAlaProThrLeuTrpAlaArgMetIleLeuMetThrHisPhePheSerValLeuIle
 8161 TTTGCCCTCACACTGTGGCGAGGATGATACTGATGACCCATTCTTAGCGTCCTTATA
 AACGGGGGTGTGACACCCGCTCTACTATGACTACTGGTAAGAAAATCGCAGGAATAT
 AlaArgAspGlnLeuGluGlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIle
 8221 GCCAGGGACCAAGCTTGACAGGCCCTCGATTGGAGATCTACGGGGCTGCTACTCCATA
 CGGTCCCTGGTCGAACCTGTCCGGAGCTAACGCTCTAGATGCCCCGGACGATGAGGTAT
 GluProLeuAspLeuProProIleIleGlnArgLeu
 8281 GAACCACCTGATCTACCTCCAATCATTCAAAGACTC
 CTTGGTGAACTAGATGGAGGTTAGTAAGTTCTGAG

-319 CACTCCACCATGAATCACTCCCCGTGAGGAACACTACTGTCTTCACGCAGAAAGCGTCTAG
 GTGAGGTGGTACTTAGTGAGGGACACTCCTTGATGACAGAAAGTGCCTTCAGCAGATC
 -259 CCATGGCGTTAGTATGAGTGTCTGCAGCCTCCAGGACCCCCCTCCGGGAGAGCCATA
 GGTACCGCAATCATACTCACAGCACGTCGGAGGTCTGGGGGAGGGCCCTCTCGGTAT
 -199 GTGGTCTGCCAACCGGTGAGTACACCGGAATTGCCAGGACGCCGGTCTTCTGG
 CACCAAGACGCCCTGGCACTCAITGCCCTAACGGTCTGGCCAGGAAAGAACCT
 -139 TCAACCCGCTCAATGCTGGAGATTGGCGTGCCCCCGAACAGACTGCTAGCCGAGTAGT
 AGTTGGCGAGTTACGGACCTCTAACCCGCACGGGGCGTCTGACGATCGGCTCATCA
 - 79 GTTGGGTGGAAAGGCCCTTGCTGAGTGGCTGATAGGGCTTGCGAGTGCCCCGGAG
 CAACCCAGCGTTCCGGAACACCATGACGGACTATCCCACGACGCTCACGGGGCCCTC
 - 19 GTCTCGTAGACCGTGCACC
 CAGAGCATCTGGCACGTGG

Arg Thr

MetSerThrAsnProLysProGlnLysAsnLysArgAsnThrAsnArgArgProGln
 1 ATGAGCAAGAATCCTAAACCTCAAAAAAAACAAACGTAACACCAACCGTCGCCACAG
 TACTCGTCTAGGATTGGAGTTTTTTTTGTTGCATTGTTGGCTGGCAGCGGGTGTC
 AspValLysPheProGlyGlyGlyGlnIlevalGlyGlyValTyrLeuLeuProArgArg
 61 GACGTCAGTTCCCGGGTGGCGGTAGATCGTTGGAGTTACTTGTGCGCGCAGG
 CTGCAGTTCAAGGGCCACCGCCAGTCTAGCAACCACCTCAAATGAACAACGGCGCGTCC
 GlyProArgLeuGlyValArgAlaThrArgLysThrSerGluArgSerGlnProArgGly
 121 GGGCCTAGATTGGGTGTGCGCGCGACGAGAAAGACTTCCGAGCGCTCGCACCTCGAGGT
 CGGGATCTAACCCACACGCGCGCTGCTCTTCTGAAGGCTCCACGCGTGGAGCTCCA
 ArgArgGlnProIleProLysAlaArgArgProGluGlyArgThrTrpAlaGlnProGly
 181 AGACGTCAGCCTATCCCCAAGGCTCGTCGGCCGAGGGCAGGACCTGGCTCAGCCCCGG
 TCTGCAGTCGGATAGGGTTCCGAGCAGCGGGCTCCGTCAGGACGGAGTCGGGCCC
 TyrProTrpProLeuTyrGlyAsnGluGlyCysGlyTrpAlaGlyTrpLeuLeuSerPro
 241 TACCCCTGGCCCTCTATGGCAATGAGGGCTGCGGGTGGCCGGATGGCTCCTGCTCTCC
 ATGGGAACCGGGGAGATAACCGTTACTCCGACGCCACCCGGCTACCGAGGGACAGAGGG
 ArgGlySerArgProSerTrpGlyProThrAspProArgArgSerArgAsnLeuGly
 301 CGTGGCTCTGGCCTAGCTGGGGCCCCACAGACCCCGCGTAGGTCGCGCAATTGGGT
 GCACCGAGAGCGGGATCGACCCGGGTCTGGGGCCGATCCAGCGCGTTAAACCCA
 LysValIleAspThrLeuThrCysGlyPheAlaAspLeuMetGlyTyrIleProLeuVal
 361 AAAGGTCACTGATACCCCTACGTGCGCTTCGCCACCTCATGGGTACATACCGCTCGTC
 TTCCAGTAGTATGGGAATGCACTGGCAAGCGCTGGAGTACCCCATGTTATGGCGAGCAG
 GlyAlaProLeuGlyGlyAlaAlaArgAlaLeuAlaHisGlyValArgValLeuGluAsp
 421 GGCGCCCCCTTGGAGGGCGCTGCCAGGGCCCTGGCGCATGGCTCCGGTTCTGGAAAGAC
 CCCGGGGAGAACCTCCCGCACGGTCCCGGACCGCGTACCGCAGGCCAACCTCTG

Thr

GlyValAsnTyrAlaThrGlyAsnLeuProGlyCysSerPheSerIlePheLeuLeuAla
 481 GGCGTGAACATGCAACAGGGAACCTTCCTGGTGTGCTCTTCTATCTTCTCTGGCC
 CGCACTTGATACGTTGCTCCCTGGAGGACCAACGAGAAAGAGATAGAAGGAAGACCGG
 LeuLeuSerCysLeuThrValProAlaSerAlaTyrGlnValArgAsnSerThrGlyLeu
 541 CTGCTCTCTGCTTGACTGTGCCCCCTCGGCCTACCAAGTGCAGACTCCACGGGGCTT
 GACGAGAGAACGAACTGACACGGCGAAGCCGGATGGTCACTCGCTGAGGTGCCCCGAA
 TyrHisValThrAsnAspCysProAsnSerSerIleValTyrGluAlaAlaAspAlaIle
 601 TACCACGTCACCAATGATTGCCCTAACTCGAGTATTGTGTACGAGGGCGGATGCCATC
 ATGGTGCACTGGTTACTAACGGGATIGAGCTCATACACATGCTCCGCCGCTACGGTAG
 LeuHisThrProGlyCysValProCysValArgGluGlyAsnAlaSerArgCysTrpVal
 661 CTGCACACTCGGGGTGCGTCCCTGCGTTCTGAGGGCAACGCCCTCGAGGTGTTGGGTG
 GACGTGTGAGGCCCCACGCAAGGAAACGCAAGCAGCTCCGTTGCGGAGCTCCACACCCAC

Fig. 17-1

721 AlaMetThrProThrValAlaThrArgAspGlyLysLeuProAlaThrGlnLeuArgArg
 GCGATGACCCCTACGGTGGCCACCAGGGATGCCAAACTCCCCCGQACCGCTTCGACGT
 CGCTACTGGGATGCCACCGTGGTCCCTACGTTGAGGGCGCTGCCTGAAGCTGCA

 781 HisIleAspLeuLeuValGlySerAlaThrLeuCysSerAlaLeuTyrValGlyAspLeu
 CACATCGATCTGCTTGTCGGGAGCGCACCCCTCTGTTCTGGCCCTCTACGTGGGGGACCTA
 GTGTAAGCTAGACGAAACAGCCCTCGCGGTGGAGACAAAGCCGGAGATGCACCCCTGGAT

 841 CysGlySerValPheLeuValGlyGlnLeuPheThrPheSerProArgArgHisTrpThr
 TGCAGGGTCTGCTTTCTTGTGCGCCAATGTTACCTCTCTCCAGGCGCACTGGACG
 ACGCCCAGACAGAAAGAACAGCCGGTTGACAAGTGGAAAGAGAGGGTCCCGGGTACCTGC

 901 ThrGlnGlyCysAsnCysSerIleTyrProGlyHisIleThrGlyHisArgMetAlaTrp
 ACGCAAGGTTGCAATTGCTCTATCTATCCCGGCCATATAACGGTCACCGCATGGCATGG
 TGCCTTCCAACGTTAACGAGATAGATAGGGCCGGTATATTGCCACTGGCGTACCGTACC

 Val
 961 AspMetMetAsnTrpSerProThrThrAlaLeuValMetAlaGlnLeuLeuArgIle
 GATATGATGATGAACTGGTCCCCTACGACGGCGTGGTAATGGCTCAGCTGCTCCGGATC
 CTATACTACTACTTGACCAGGGATGCTGGCGCAACCATTACCGACTGACGAGGCCTAG

 1021 ProGlnAlaIleLeuAspMetIleAlaGlyAlaHisTrpGlyValLeuAlaGlyIleAla
 CCACAAGCCATCTGGACATGATCGCTGGTGTCACTGGGGAGTGCCTGGCGGGCATAGCG
 GGTGTTCGGTAGAACCTGTACTAGCGACCRCGAGTGACCCCTCAGGACCGCCGTATCGC

 1081 TyrPheSerMetValGlyAsnTrpAlaLysValLeuValLeuLeuLeuPheAlaGly
 TATTTCTCCATCTGGTGGGGAACTGGCGAAGGTCTGGTAGTGCCTGCTATTGCGCGC
 ATAAAGAGGTACCCACCCCTTGACCCGCTTCCAGGACCATCACGACGACGATAAACGGCCG

 1141 ValAspAlaGluThrHisValThrGlyGlySerAlaGlyHisThrValSerGlyPheVal
 GTCGACGCGGAAACCCACGTCAACGGGGAAAGTGCCTGGCCACACTGTTCTGGATTGTT
 CAGCTGCCCTTGTGGTGAGTGGCCCTTACGGCCGGTGACACAGACTAAACAA

 1201 SerLeuLeuAlaProGlyAlaLysGlnAsnValGlnLeuIleAsnThrAsnGlySerTrp
 AGCCTCCTCGCACCGGCCAACGGCGCAAGCAGAACGTCAGCTGATCAACACCAACGGCAGTGG
 TCGGAGGAGCGTGGTCCCGGTTCTGAGCTGACTAGTTGTGGTCCGTCAACC

 1261 HisLeuAsnSerThrAlaLeuAsnCysAsnAspSerLeuAsnThrGlyTrpLeuAlaGly
 CACCTCAATAGCACGGCCCTGAACGTTGATAGCTCAACACCCGCTGGTGAGCAGGG
 GTGGAGTTATCGTGCAGGACTTGACGTTACTATCGGAGTTGTGGCCGACCAACCGTCCC

 LeuPheTyrHisHisLysPheAsnSerSerGlyCysProGluArgLeuAlaSerCysArg
 1321 CTTTCTATCACCAAGTTCAACTCTCAGGCTGTCCTGAGAGGCTAGCCAGCTGCCGA
 GAAAAGATAGTGGTGTCAAGTTGAGAAGTCCGACAGGACTCTCCGATGGTCGACGGCT

 ProLeuThrAspPheAspGlnGlyTrpGlyProIleSerTyrAlaAsnGlySerGlyPro
 1381 CCCCTTACCGATTTGACCAAGGGCTGGGCCCTATCAGTTATGCCAACGGAAAGCGGGCCC
 GGGGAATGCCAAAACTGGTCCCACCCGGGATAGTCATAACGGTTGCCCTCGCCGGGG

 1441 AspGlnArgProTyrCysTrpHisTyrProProLysProCysGlyIleValProAlaLys
 GAACAGCGCCCTACTGCTGGCACTACCCCCCAAACCTTGCGGTATTGTGCCCGCGAAG
 CTGGTCGCGGGATGACGACCGTGTGGGGTTTGGAACGCCATAACACGGCGCTTC

 1501 SerValCysGlyProValTyrCysPheThrProSerProValValGlyThrThrAsp
 AGTGTGTGGTCCGGTATATTGCTCACTCCCAGCCCGTGGTGGTGGGAACGACCGAC
 TCACACACACCGGCCATATAACGAGTGAGGGTCCGGGACCCACCCCTGCTGGCTG

 1561 ArgSerGlyAlaProThrTyrSerTrpGlyGluAsnAspThrAspValPheValLeuAsn
 AGGTGGCGCCGCCACCTACAGCTGGGTGAAAATGATACGGACGTCTTCGTCCTTAAC
 TCCAGCCCGCGCGGGTGGATGTCGACCCACTTTACTATGCCCTGAGAAGCAGGAATTG

 1621 AsnThrArgProProLeuGlyAsnTrpPheGlyCysThrTrpMetAsnSerThrGlyPhe
 AATACCAAGGCCACCGCTGGCAATTGTTGGTTGTACCTGGATGAACCTCAACTGGATTC
 TTATGGTCCGGTGGCGACCGTTAACCAAGCCAACATGGACCTACTTGAGTTGACCTAAG

Fig. 17-2

1681 ThrLysValCysGlyAlaProProCysValIleGlyGlyAlaGlyAsnAsnThrLeuHis
 ACCAAAGTGTGCGGAGCGCCTCCTTGTGTCATCGGAGGGCGGGCAACAAACACCCGTGCAC
 TGGTTTACACGCCCTCGCGGAGGAACACAGTAGCCTCCCCGCCGTGTTGTGGGACGTG

1741 CysProThrAspCysPheArgLysHisProAspAlaThrTyrSerArgCysGlySerGly
 TGCCCCACTGATTGCTTCGCAAGCATCCGGACGCCACATACTCTCGGTGCGGCTCCGGT
 ACGGGGTGACTAACGAAGGCAGTCGTAGGCCTGCGGTGTATGAGAGCCACGCCAGGCCA

Leu

1801 ProTrpIleThrProArgCysLeuValAspTyrProTyrArgLeuTrpHisTyrProCys
 CCCTGGATCACACCCAGGTGCGCTGGTGCACCTACCCGTATAGGCTTGGCATTATCCTTGT
 GGGACCTAGTGTGGGTCCACGGACCAGCTGATGGCATATCCGAAACCGTAATAGGAACA

1861 ThrIleAsnTyrThrIlePheLysIleArgMetTyrValGlyGlyValGluHisArgLeu
 ACCATCAACTACACCATAAATTTAAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGCTG
 TGGTAGTTGATGTGGTATAAATTTAGTCCTACATGCACCCCTCCCCAGCTTGTGTCCGAC

1921 GluAlaAlaCysAsnTrpThrArgGlyGluArgCysAspLeuGluAspArgAspArgSer
 GAAGCTGCCTGCAACTGGACGCGGGCGAACGTTGCGATCTGGAAGACAGGGACAGGTCC
 CTCGACGGACGTTGACCTGCGCCCCGCTTGCACGCTAGACCTCTGTCCCTGTCCAGG

1981 GluLeuSerProLeuLeuLeuThrThrGlnTrpGlnValLeuProCysSerPheThr
 GAGCTCAGCCCGTTACTGCTGACCAACTACACAGTGGCAGGTCCCTCCGTGTTCCCTACAA
 CTCGAGTCGGGCAATGACGACTGGTGTAGTCACCGTCAGGAGGGCACAAAGGAAGTGT

2041 ThrLeuProAlaLeuSerThrGlyLeuIleHisLeuHisGlnAsnIleValAspValGln
 ACCCTACCAGCCTGTCCACCGCCCTCATCCACCTCCACAGAACATTGTGGACGTGCAG
 TGGGATGGTCGGAACAGGTGGCCGGAGTAGGTGGAGGTGGTCTTGTAAACACCTGCACGTC

2101 TyrLeuTyrGlyValGlySerSerIleAlaSerTrpAlaIleLysTrpGluTyrValVal
 TACTTGTACGGGGTGGGGTCAAGCATCGCGTCTGGGCCATTAAGTGGGAGTACGTCGTT
 ATGAACATGCCCAACCCAGTCGTAGCGCAGGACCCGGTAATTACCCCTCATGCAGCAA

2161 LeuLeuPheLeuLeuLeuAlaAspAlaArgValCysSerCysLeuTrpMetMetLeuLeu
 CTCCCTTCCTCTGCTTGCAGACGCGCGCTGTGCTCTGCTTGTGGATGATGCTACTC
 GAGGACAAGGAAGACGAACGTCTGCGCGCAGACGAGGACAAACCTACTACGATGAG

2221 IleSerGlnAlaGluAlaAlaLeuGluAsnLeuValIleLeuAsnAlaAlaSerLeuAla
 ATATCCAAGCGGAGGCGCTTGGAGAACCTCGTAATACTTAATGCGCATCCCTGGCC
 TATAGGGTTGCCCTCCGCCGAAACCTTGGAGCATTATGAATTACGTCGTAGGGACCGG

2281 GlyThrHisGlyLeuValSerPhaLeuValPhePheCysPheAlaTrpTyrLeuLysGly
 GGGACGCACGGTCTTGTATCCTCTCGTGTCTGCTTGCATGGTATTGAAGGGT
 CCCTGCGTGCAGAACATAGGAAGGACACAAGAACGTACCAAACTTCCC

2341 LysTrpValProGlyAlaValTyrThrPheTyrGlyMetTrpProLeuLeuLeuLeu
 AAGTGGGTGCCCGAGCGGTCTACACCTCTACGGGATGTGGCCTCTCCTGCTCCTG
 TTCACCCACGGCCTCGCAGATGTGGAGATGCCCTACACCGAGAGGAGGACGAGGAC

2401 LeuAlaLeuProGlnArgAlaTyrAlaLeuAspThrGluValAlaAlaSerCysGlyGly
 TTGGCGTTGCCCAAGCGGGCGTACCGCGTGGACACGGAGGTGGCCCGTGTGGCGGT
 AACCGCAACGGGGTCGCCCGCATGCGCAGCTGTGCCTCACCGCGCAGCACACCGCCA

2461 ValValLeuValGlyLeuMetAlaLeuThrLeuSerProTyrTyrLysArgTyrIleSer
 GTTGTCTCGTCGGGTTGATGGCGCTGACTCTGTCACCATATTACAAGCGCTATATCAGC
 CAACAAGAGCAGCCCCACTACCGCGACTGAGACAGTGGTATAATGTCGCGATATAGTCG

Asn

2521 TrpCysLeuTrpTrpLeuGlnTyrPheLeuThrArgValGluAlaGlnLeuHisValTrp
 TGGTGTGTTGTGGCTTCAGTATTTCTGACAGAGTGGAAAGCGCAACTGCACGTGTGG
 ACCACGAACACCACCGAAGTCATAAAAGACTGGTCTCACCTCGCGTTGACGTGCACACC

2581 IleProProLeuAsnValArgGlyGlyArgAspAlaValIleLeuLeuMetCysAlaVal
 ATTCCCCCCCCTAACGTCCGAGGGGGCGCGACGCCGTACCTTACTCATGTGTGCTGTA
 TAAGGGGGAGTTGCAGGCTCCCCCGCGTGCAGTAGAATGAGTACACACGACAT

Fig. 17-3

2641 HisProThrLeuValPheAspIleThrLysLeuLeuLeuAlaValPheGlyProLeuTrp
 CACCCGACTCTGGTATTGACATCACCAAATTGCTGCTGGCGTCTTCGGACCCCTTGG
 GTGGGCTGAGACCATAAACTGTAGTGGTTAACGACGACCGCAGAAGCCTGGGAAACC

 2701 IleLeuGlnAlaSerLeuLeuLysValProTyrPheValArgValGlnGlyLeuLeuArg
 ATTCTCAAGCCAGTTGCTAAAGTACCTACTTGCGCGTCCAAGGCCTTCGCC
 TAAGAAGTTCGGTCAAACGAATTTCATGGGATGAAACACGCCAGGTTCCGGAAGAGGCC

 2761 PheCysAlaLeuAlaArgLysMetIleGlyGlyHisTyrValGlnMetValIleIleLys
 TTCTGCGCGTTAGCGCGGAAGATGATCGGAGGCCATTACGTCAAATGGTCATCATTAAG
 AAGACCGCGAATCGCGCTTCTACTAGCCTCCGGTAATGCACGTTACCACTAGTAATT

 2821 LeuGlyAlaLeuThrGlyThrTyrValTyrAsnHisLeuThrProLeuArgAspTrpAla
 TTAGGGCGCTTACTGGCACCTATGTTATAACCATCTCACTCCTCTCAGGACTGGGCG
 AATCCCCCGAATGACCGTGGATACAAATATTGGTAGAGTGAGGAGAACCCCTGACCCGC

 2881 HisAsnGlyLeuArgAspLeuAlaValAlaValGluProValValPheSerGlnMetGlu
 CACAACGGCTTGCGAGATCTGGCCGTGGCTGTAGAGCCAGTCGTCTCTCCAAATGGAG
 GTGTTGCCAACGCTCTAGACCGCACCGACATCTGGTCAGCAGAACAGGGTTACCTC

 2941 ThrLysLeuIleThrTrpGlyAlaAspThrAlaAlaCysGlyAspIleIleAsnGlyLeu
 ACCAACGCTCATCAGTGGGGGGCAGATAACGCCGCGTGGCTGACATCATCAACGGCTTG
 TGGTTCGAGTAGTGCACCCCCCGTCTATGGCGCGCACGCCACTGTAGTAGTTGCCAAC

 3001 ProValSerAlaArgArgGlyArgGluIleLeuLeuGlyProAlaAspGlyMetValSer
 CCTGTTTCCGCCGAGGGGGGGAGATACTGCTCGGGCCAGCCGATGGAATGGTCTCC
 GGACAAAGGCCGGCGTCCCCGCCCTCTATGACGAGCCGGTGGCTACCTTACCAAGAGG

 3061 LysGlyTrpArgLeuLeuAlaProIleThrAlaTyrAlaGlnGlnThrArgGlyLeuLeu
 AAGGGGTGGAGGTTGCTGGCGCCCATCACGGCGTACGCCAGCAGAACAGGGGCCCTCTA
 TTCCCCACCTCCAACGACCGCGGGTAGTGGCGCATGGGGTGTCTGTTCCCCGGAGGAT

 3121 GlyCysIleIleThrSerLeuThrGlyArgAspLysAsnGlnValGluGlyGluValGln
 GGGTGCATAATCACAGCCTAAGTGGCGGGACAAAAACCAAGTGGAGGGTGAGGTCCAG
 CCCACGTATTAGTGGTCGGATTGACCGGCCCTGTTGGTACCTCCACTCCAGGTC

 3181 IleValSerThrAlaAlaGlnThrPheLeuAlaThrCysIleAsnGlyValCysTrpThr
 ATTGTGTCAGCTGCCAACCTTCCTGGCAACGTGCATCAATGGGGTGTGCTGGACT
 TAACACAGTTGACGACGGTTGGAAAGGACCGTGGCACGTAGTTACCCACACGACCTGA

 3241 ValTyrHisGlyAlaGlyThrArgThrIleAlaSerProLysGlyProValIleGlnMet
 GTCTACCACGGGGCGGAACGAGGACCATCGCGTACCCAGGGTCTGTCATCCAGATG
 CAGATGGTGCCTGGCTGGTAGCGCAGTGGGTCCTCAGACTAGGTCTAC

 3301 Ser Thr
 TyrThrAsnValAspGlnAspLeuValGlyTrpProAlaProGlnGlySerArgSerLeu
 TATACCAATGTAGACCAAGACCTTGTGGCTGGCCGCTCCGCAAGGTAGCCGCTCATG
 ATATGGTTACATCTGGTCTGGAACACCCGACCGGGCGAGGCGTCCATGGCGAGTAAC

 3361 ThrProCysThrCysGlySerSerAspLeuTyrLeuValThrArgHisAlaAspValIle
 ACACCCACTGCACTTGGCGCTCTCGGACCTTACCTGGTACGAGGCACGCCGATGTCATT
 TGTGGGACGTGAACGCCGAGGAGCCTGGAAATGGACCACTGCTCCGTGGCTACAGTAA

 3421 ProValArgArgArgGlyAspSerArgGlySerLeuLeuSerProArgProIleSerTyr
 CCCGTGCGCCGGCGGGGTGATAGCAGGGGCAGCCTGCTGTCGCCCCGGCCCATTCTAC
 GGGCACGCCGCCCCACTATCGTCCCCGCGACAGCAGGGCCGGTAAAGGATG

 3481 LeuLysGlySerSerGlyGlyProLeuLeuCysProAlaGlyHisAlaValGlyIlePhe
 TTGAAAGGCTCCTCGGGGGTCCGCTGTTGTGCCCCCGGGGCACGCCGTGGCATATT
 AACTTCCGAGGAGCCCCCAGGCGACAACACGGGGCGCCCCGTGGCACCCGTATAAA

 3541 ArgAlaAlaValCysThrArgGlyValAlaLysAlaValAspPheIleProValGluAsn
 AGGGCCGCGGTGTGCAACCGTGGAGTGGCTAAGGCCGTGGACTTATCCCTGTGGAGAAC
 TCCCGCGGCCACACGTGGCACCTCACCGATTCCGCCACCTGAAATAGGGACACCTCTTG

Fig. 17-4

3601 LeuGluThrThrMetArgSerProValPheThrAspAsnSerSerProProValValPro
 CTAGAGACAAACCATGAGGTCCCCGGTTCACGGATAACTCCTCTCACCAAGTAGTGCCC
 GATCTCTGTTGGTACTCCAGGGGCACAAGTGCCTATTGAGGAGAGGTGGTCATCACGGG

 3661 GlnSerPheGlnValAlaHisLeuHisAlaProThrGlySerGlyLysSerThrLysVal
 CAGAGCTTCAGGTGGCTCACCTCATGCTCCCACAGGCAGCGCAAAGCACCAAGGTC
 GTCTCGAAGGTCCACCGAGTGGAGGTACGAGGGTGTCCGTGCCGTTTCGTGGTCCAG

 3721 ProAlaAlaAlaTyrAlaAlaGlnGlyTyrLysValLeuValLeuAsnProSerValAlaAla
 CCGGCTGCATATGCACCTCAGGGCTATAAGGTGCTAGTACTCAACCCCTCTGTTGCTGCA
 GGCGACGTATACGTCGACTCCGATATTCCACGATCATGAGTTGGGAGACAAACGACGT

 3781 Leu
 ThrLeuGlyPheGlyAlaTyrMetSerLysAlaHisGlyIleAspProAsnIleArgThr
 ACACTGGGCTTGGTGCTTACATGTCCAAGGCTCATGGGATCGATCTAACATCAGGACC
 TGTGACCCGAAACCACGAATGTACAGGTTCCGAGTACCCCTAGCTAGGATTGTAGTCCTGG

 3841 GlyValArgThrIleThrThrGlySerProIleThrTyrSerThrTyrGlyLysPheLeu
 GGGGTGAGAACAAATTACCACTGGCAGCCCCATCACGTACTCCACCTACGGCAAGTTCCTT
 CCCCACTCTTGTAAATGGTGACCGTGGGGTAGTGCATGAGGTGGATGCCGTTCAAGGAA

 3901 AlaAspGlyGlyCysSerGlyGlyAlaTyrAspIleIleIleCysAspGluCysHisSer
 GCCGACGGCGGGTGCTCGGGGGGGCGCTTATGACATAATAATTGTGACGAGTGCCACTCC
 CGGCTGCCGCCAACGAGCCCCCGCGAATACTGTATTAAACACTGCTCACGGTGAGG

 3961 ThrAspAlaThrSerIleLeuGlyIleGlyThrValLeuAspGlnAlaGluThrAlaGly
 ACGGATGCCACATCCATCTGGGCATCGGCACTGTCCCTGACCAAGCAGAGACTGCGGGG
 TGCCTACGGTGTAGGTAGAACCGTAGCCGTGACAGGAACCTGGTCTCTGACGCCCC

 4021 AlaArgLeuValValLeuAlaThrAlaThrProProGlySerValThrValProHisPro
 GCGAGACTGGTTGTGCTGCCACCGCCACCCCTCCGGCTCCGTACTGTGCCCCATCCC
 CGCTCTGACCAACACGAGCGGTGGCGGTGGGAGGCCAGGCAGTACACGGGTAGGG

 4081 AsnIleGluGluValAlaLeuSerThrThrGlyGluIleProPheTyrGlyLysAlaIle
 AACATCGAGGAGGGTTGCTCTGTCCACCACCGGAGAGATCCCTTTACGGCAAGGCTATC
 TTGTAGCTCCCAACGAGACAGGTGGTGGCTCTAGGGAAAAATGCCGTTCCGATAG

 4141 ProLeuGluValIleLysGlyGlyArgHisLeuIlePheCysHisSerLysLysCys
 CCCCTCGAAGTAATCAAGGGGGGGAGACATCTCATCTTCGTATTCAAAGAAGAAGTGC
 GGGGAGCTCATTAGTCCCCCCCCTCTGTAGAGTAGAACAGTAAGTTCTTCACG

 4201 AspGluLeuAlaAlaLysLeuValAlaLeuGlyIleAsnAlaValAlaTyrTyrArgGly
 GACGAACTCGCCGAAAGCTGGTCCGATTGGGCATCAATGCCGTGGCTACTACCGCGGT
 CTGCTTGAGCGCGTTCGACCAGCGTAACCGTAGTTACGGCACCGATGATGGCGCCA

 4261 LeuAspValSerValIleProThrSerGlyAspValValValAlaThrAspAlaLeu
 CTTGACGTGTCGCTCATCCGACCAGCGGGCAGTGTGCGTGGCAACCGATGCCCTC
 GAACTGCACAGGCAGTAGGGCTGGTCGCCGCTAACACAGCAGCACCGTTGGCTACGGGAG

 4321 Tyr
 MetThrGlyTyrThrGlyAspPheAspSerValIleAspCysAsnThrCysValThrGln
 ATGACCGGCTATACCGGCACTTCGACTCGGTGATAGACTGCAATACGTGTCAACCCAG
 TACTGGCCGATATGGCCGCTGAAGCTGAGCCACTATCTGACGTTATGCACACAGTGGGTC

 4381 Ser
 ThrValAspPheSerLeuAspProThrPheThrIleGluThrIleThrLeuProGlnAsp
 ACAGTCGATTTCAGCCTGACCCCTACCATGAGACAATCACGCTCCCCCAGGAT
 TGTCAAGCTAAAGTCGGAACGTGGATGGAAGTGGTAACCTGTGAGCTGACGTTATGCACACAGTGGGTC

 4441 AlaValSerArgThrGlnArgArgGlyArgThrGlyArgGlyLysProGlyIleTyrArg
 GCTGTCTCCCGCACTAACGTCGGGGCAGGACTGGCAGGGGGAGCCAGGCATCTACAGA
 CGACAGAGGGCGTGAGTGCAGCCCCGTCCTGACCGTCCCCCTCGGTCCGTAGATGTCT

 4501 PheValAlaProGlyGluArgProSerGlyMetPheAspSerSerValLeuCysGluCys
 TTTGTGGCACCGGGGGAGCGCCCCCTCCGGCATGTTGCACTCGTCCGTCTGTGAGTGC
 AACACCGTGGCCCCCTCGCGGGAGGCCGTACAAGCTGAGCAGGCAGGAGACACTCACG

Fig. 17-5

4561 TyrAspAlaGlyCysAlaTrpTyrGluLeuThrProAlaGluThrThrValArgLeuArg
 TATGACGCAGGCTGTGCTGGTATGAGCTCACGCCGCCGAGACTACAGTTAGGCTACGA
 ATACTGCGTCCGACACGAACCATACTCGAGTGCAGGGCTCTGATGTCAATCCGATGCT

4621 AlaTyrMetAsnThrProGlyLeuProValCysGlnAspHisLeuGluPheTrpGluGly
 GCGTACATGAACACCCCCGGGGCTTCCCCTGTGCCAGGACCATTTGAATTGGGAGGGC
 CGCATGTACTTGTGGGGCCCGAAGGGCACACGGTCCTGGTAGAACTAAAACCTCCCG

4681 ValPheThrGlyLeuThrHisIleAspAlaHisPheLeuSerGlnThrLysGlnSerGly
 GTCTTACAGGCCTCACTCATATAGATGCCACTTCTATCCCAGACAAAGCAGAGTGGG
 CAGAAATGTCCGGAGTGAGTATATCTACGGGTGAAAGATAGGGTCTGTTCGTCTCACCC

4741 GluAsnLeuProTyrLeuValAlaTyrGlnAlaThrValCysAlaArgAlaGlnAlaPro
 GAGAACCTTCCTTACCTGGTAGCGTACCAAGCCACCGTGTGCGCTAGGGCTCAAGCCCCT
 CTCTTGAAGGAATGGACCATCGCATGGTGGCACACGGCAGTCCGAGTTGGGAG

4801 ProProSerTrpAspGlnMetTrpLysCysLeuIleArgLeuLysProThrLeuHisGly
 CCCCCATCGTGGGACCAAGATGTTGAAAGTGTGTTGATTGCCCTCAAGCCCACCCCTCATGGG
 GGGGGTAGCACCCTGGTCTACACCTTACAACAACTAACGGAGTTGGGTGGGAGGTACCC

4861 ProThrProLeuLeuTyrArgLeuGlyAlaValGlnAsnGluIleThrLeuThrHisPro
 CCAACACCCCTGCTATACAGACTGGGCGCTGTTCAGAATGAAATCACCTGACGCACCCA
 GGTTGTGGGACGATATGTCTGACCCGGACAAAGTCTTACTTAAAGTGGACTGCGTGGGT

4921 ValThrLysTyrIleMetThrCysMetSerAlaAspLeuGluValValThrSerThrTrp
 GTCACCAAATACATCATGACATGTCGGCCGACCTGGAGGTGTCACGAGCACCTGG
 CAGTGGTTATGTAGTACTGTACAGCCGGCTGGACCTCCAGCAGTGTCTCGTGGACC

4981 ValLeuValGlyGlyValLeuAlaAlaLeuAlaAlaTyrCysLeuSerThrGlyCysVal
 GTGCTCGTTGGCGCGTCTGGCTGCTTGGCCCGTATTGCCCTGTCAACAGGCTGCGTG
 CACGAGCAACCGCCGAGGACCGACGAAACCGGCGATAACGGACAGTTGTCCGACGCAC

5041 ValIleValGlyArgValValLeuSerGlyLysProAlaIleProAspArgGluVal
 GTCATAGTGGGCAGGGTCGTCTGTCCGGGAAGCCGGCAATCATACCTGACAGGGAAAGTC
 CAGTATCACCCGTCAGCAGAACAGGCCCTCGGCCGTTAGTATGGACTGTCCCTTCAG

5101 LeuTyrArgGluPheAspGluMetGluGluCysSerGlnHisLeuProTyrIleGluGln
 CTCTACCGAGAGTCGATGAGATGGAAGAGTGCTCTCAGCACTACCGTACATCGAGCAA
 GAGATGGCTCTCAAGCTACTCTACCTCTCACGAGAGTCGTGAATGGCATGTAGCTCGTT

5161 GlyMetMetLeuAlaGluGlnPheLysGlnLysAlaLeuGlyLeuLeuGlnThrAlaSer
 GGGATGATGCTCGCCGAGCAGTTCAAGCAGAAGGCCCTCGGCCCTCGCAGACCGCGTCC
 CCCTACTACGAGCGGCTCGTCAAGTTGCTTCCGGGAGCCGGAGGACGTCTGGCGCAGG

5221 ArgGlnAlaGluValIleAlaProAlaValGlnThrAsnTrpGlnLysLeuGluThrPhe
 CGTCAGGCAGAGGTTATGCCCTGCTGCTCAGACCAACTGGAAAAACTCGAGACCTTC
 GCAGTCCTGCTCCAATAGCGGGACGACAGGTCTGGTTGACCGTTTGAGCTCTGGAAAG

5281 TrpAlaLysHisMetTrpAsnPheIleSerGlyIleGlnTyrLeuAlaGlyLeuSerThr
 TGGCGAAGCATATGTGAACTTCATCAGTGGATACAATACTGGCAGGCTTGTCAACG
 ACCCGCTTCGTATACACCTTGAAGTAGTCACCCATGTTATGAACCGCCGAACAGTTGC

5341 LeuProGlyAsnProAlaIleAlaSerLeuMetAlaPheThrAlaAlaValThrSerPro
 CTGCCTGGTAACCCGCCATTGCTTCATTGATGGCTTTACAGCTGCTGTCACCAGCCCA
 GACGGACCATTGGGGCGGTACGAAGTAACGAAACTACCGAAAATGTCGACGACAGTGGTCGGGT

5401 LeuThrThrSerGlnThrLeuLeuPhaAsnIleLeuGlyGlyTrpValAlaAlaGlnLeu
 CTAACCCTAGCCAAACCCCTCTCTTCAACATATTGGGGGGTGGGTGGCTGCCAGCTC
 GATTGGTGATCGGTTGGGAGGAGAAGTTGTATAACCCCCCACCACCGACGGGTCGAG

5461 AlaAlaProGlyAlaAlaAlaThrAlaPheValGlyAlaGlyLeuAlaGlyAlaIleGly
 GCCGCCCGGCTGGCGCTACTGCCTTGCGCTGGCTAGCTGGCGCCGACCGATCGACGG
 CGCGGGGGCCACGGCGATGACGGAAACACCCCGCAGCGAACATCGACCGCGGGTAGCCG

SerValGlyLeuGlyLysValLeuIleAspIleLeuAlaGlyTyrGlyAlaGlyValAla

Fig. 17-6

5521 AGTGTGGACTGGGAAGGTCTCATAGACATCTGCAGGGTAIGCGCGGGCGTGGCG
 TCACAACCTGACCCCTTCAGGAGTATCTGTAGAACGTCCCATACCGCGGCCGACCGC
 Gly
 ,581 GlyAlaLeuValAlaPheLysIleMetSerGlyGluValProSerThrGluAspLeuVal
 GGAGCTTTGTGGCATTCAAGATCATGAGCGGTGAGGTCCCCTCCACGGAGGACCTGGTC
 CCTCGAGAACACCGTAAGTTCTAGTACTGCCACTCCAGGGAGGTGCTCCTGGACCAG
 AsnLeuLeuProAlaIleLeuSerProGlyAlaLeuValValGlyValValCysAlaAla
 5641 AATCTACTGCCGCCATCTCTGCCCGAGCCCTCGTAGTCGGGTGGTCTGTGCAGCA
 TTAGATGACGGCGGTAGGAGAGCGGGCCTCGGGAGCATCAGCCGACCCAGACACGTCGT
 IleLeuArgArgHisValGlyProGlyGluGlyAlaValGlnTrpMetAsnArgLeuIle
 5701 ATACTGCGCCGGCACGTGGCCCGGGCGAGGGGGCAGTGAGTGGATGAACCGGCTGATA
 TATGACGCGGCCGTGCAACCGGGCCCGTCCCCCGTACGTACCTACTTGGCCGACTAT
 AlaPheAlaSerArgGlyAsnHisValSerProThrHisTyrValProGluSerAspAla
 5761 GCCTTCGCTCCGGGGAAACCATGTTCCCCCACGCACTACGTGCCGGAGAGCGATGCA
 CGGAAGCGGAGGGCCCCCTGGTACAAAGGGGTGCGTGATGCACGGCTCTCGCTACGT
 HisCys
 AlaAlaArgValThrAlaIleLeuSerSerLeuThrValThrGlnLeuLeuArgArgLeu
 5821 GCTGCCCGCGTCACTGCCATACTCAGCAGCCTCACTGTAACCCAGCTCTGAGGGCACIG
 CGACGGGCGCACTGACGGTATGAGTCGGAGTGACATGGGTGAGGACTCCGCTGAC
 HisGlnTrpIleSerSerGluCysThrThrProCysSerGlySerTrpLeuArgAspIle
 5881 CACCAAGTGGATAAGCTGGAGTGTACCACTCCATGCTCCGGTTCTGGCTAAGGGACATC
 GTGGTCACCTATTGAGCCTCACATGGTGAGGTACGAGGCAAGGACCGATTCCCTGTAG
 TrpAspTrpIleCysGluValLeuSerAspPheLysThrTrpLeuLysAlaLysLeuMet
 5941 TGGGACTGGATATGCGAGGTGGAGCCTTAAGACCTGGCTAAAGCTAACGCTCATG
 ACCCTGACCTATACGCTCCACAACCTCGTCAAATTCTGGACCGATTTCGATTGAGTAC
 ProGlnLeuProGlyIleProPheValSerCysGlnArgGlyTyrLysGlyValTrpArg
 6001 CCACAGCTGCCCTGGGATCCCCTTGTGTCCTGCCAGCGCGGGTATAAGGGGGTCTGGCGA
 GGTGTCGACGGACCCCTAGGGAAACACAGGACGGTCCGCCCCATATTCCCCCAGACCGCT
 Gly
 ValAspGlyIleMetHisThrArgCysHisCysGlyAlaGluIleThrGlyHisValLys
 6061 GTGGACGGCATCATGCACACTCGCTGCCACTGTTGAGCTGAGATCACGGACATGTCAAA
 CACCTGCCGTAGTACGTGTGAGCGACGGTACACCTCGACTCTAGTGACCTGTACAGTT
 AsnGlyThrMetArgIleValGlyProArgThrCysArgAsnMetTrpSerGlyThrPhe
 6121 AACGGGACGATGAGGATCGTCGGCTCTAGGACCTGCAGGAACATGTGGAGTGGGACCTTC
 TTGCCCCGCTACTCCTAGCAGCCAGGATCCTGGACGTCTGTACACCTCACCCCTGGAAAG
 ProIleAsnAlaTyrThrThrGlyProCysThrProLeuProAlaProAsnTyrThrPhe
 6181 CCCATTAAATGCCCTACACCACGGGCCCTGTACCCCCCTCTGCCGCGAACTACACGTT
 GGGTAATTACGGATGTGGTGGCCGGGACATGGGGGAAGGACGCGGGTTGATGTGCAAG
 AlaLeuTrpArgValSerAlaGluGluTyrValGluIleArgGlnValGlyAspPheHis
 6241 GCGCTATGGAGGGTGTCTGCAGAGGAATATGTGGAGATAAGGCAGGTGGGGACTTCCAC
 CGCGATACCTCCCACAGACGTCTCTTACACCTCTATTCCGTCACCCCTGAAGGTG
 TyrValThrGlyMetThrThrAsnLeuLysCysProCysGlnValProSerProGlu
 6301 TAGTGACGGTATGACTACTGACAATCTCAAATGCCGTGCCAGGTCCATGCCCGAA
 ATGCACTGCCATACTGACTGTAGTTACGGGCACGGTCCAGGGTAGCGGGCTT
 PhePheThrGluLeuAspGlyValArgLeuHisArgPheAlaProProCysLysProLeu
 6361 TTTTCACAGAAATTGGACGGGGTGCCTACATAGGTTTGCAGCCCCCTGCAAGCCCTTG
 AAAAGTGTCTAACCTGCCACCGGGATGTATCCAACGGGGGAGCTCAGGGAAAC
 LeuArgGluGluValSerPheArgValGlyLeuHisGluTyrProValGlySerGlnLeu
 6421 CTGGGGAGGGAGGTATCAAGAGTAGGACTCCACGAATACCGGTAGGGTCGCAATT
 GACGCCCTCCATAGTAACTCATCCTGAGGTGTTATGGGCCATCCAGCGTTAAT

Fig. 17-7

6481 ProCysGluProGluProAspValAlaValLeuThrSerMetLeuThrAspProSerHis
 CCTTGCGAGCCCCGAACCGGACGTGGCCGTGTTGACGTCCATGCTCACTGATCCCTCCCCT
 GGAACGCTCGGGCTTGGCCTGCACCGCACAACTGCAGGTACGAGTGACTAGGGAGGGTA

 541 IleThrAlaGluAlaAlaGlyArgArgLeuAlaArgGlySerProProSerValAlaSer
 ATAACAGCAGGGCGGCCGGCGAAGGGTGGCGAGGGGATCACCCCCCTCTGTGGCCAGC
 TATTGTCGTCTCCGCGGGCCCGCTTCCAACCGCTCCCTAGTGGGGGAGACACCGGTG

 6601 SerSerAlaSerGlnLeuSerAlaProSerLeuLysAlaThrCysThrAlaAsnHisAsp
 TCCTCGGCTAGCCAGCTATCCGCTCCATCTCAAGGCAACTTGCACCGCTAACCATGAC
 AGGAGCCGATCGGTGCGATAGGCGAGGTAGAGACTCCGTTGAACGTGGCGATGGTACTG

 6661 SerProAspAlaGluLeuIleGluAlaAsnLeuLeuTrpArgGlnGluMetGlyGlyAsn
 TCCCCGTGATGCTGAGCTCATAGAGGCCAACCTCTATGGAGGCAGGAGATGGCGGGCAC
 AGGGGACTACGACTCGAGTATCTCCGGTTGGAGGATACCTCCGCTCTACCCGCCGTTG

 6721 IleThrArgValGluSerGluAsnLysValValIleLeuAspSerPheAspProLeuVal
 ATCACCAAGGGTTGAGTCAGAAAACAAAGTGGTATTCTGGACTCCTCGATCCGCTTGTG
 TAGTGGTCCCAACTCAGTCTTTGTTTCAACCCTAACGACTGAGGAAGCTAGGCGAACAC

 6781 AlaGluGluAspGluArgGluIleSerValProAlaGluIleLeuArgLysSerArgArg
 GCGGAGGGAGGACGAGCGGGAGATCTCGTACCCGCAAAATCTCGGGAGTCTCGGAGA
 CGCCTCCTCCTGTCGCCCCTAGAGGCATGGCGCTTTAGGGACGCCTCAGAGCCTCT

 6841 PheAlaGlnAlaLeuProValTrpAlaArgProAspTyrAsnProProLeuValGluThr
 TTTCGCCCAGGCCCTGCCCCGTTGGCGCGCCGACTATAACCCCCCGCTAGTGGAGACG
 AAGCGGGTCCGGGACGGGCAAACCGCGCCGGCTGATATTGGGGGGCGATCACCTCTGC

 6901 TrpLysProAspTyrGluProProValValHisGlyCysProLeuProProProLys
 TGGAAAAAGCCCGACTACGAAACCACCTGTTGGTCCATGGCTGTCGCTTCCACCTCCAAAG
 ACCTTTTCGGGCTGATGCTTGGTGGACACCAGGTACCGACAGGGCAAGGTGGAGGTTTC

 6961 SerProProValProProProArgLysLysArgThrValValLeuThrGluSerThrLeu
 TCCCCCTCTGTGCCCTCGGCCTCGGAAGAAGCGGACGGTGGCTCTCACTGAATCAACCTA
 AGGGGAGGACACGGAGGCGAGCCTCTCGCCTGCCACCAGGAGTGAATTAGTTGGGAT

Fig. 17-8

Ser

7021 SerThrAlaLeuAlaGluLeuAlaThrArgSerPheGlySerSerSerThrSerGlyIle
 TCTACTGCCTTGCCGAGCTCGCCACCAGAACGTTGGCAGCTCCTCAACTTCCGGCATT
 AGATGACGGAACCGGCTCGAGCGGTGGCTTCGAAACCGTCGAGGAGTTGAAGGCCGAA

 7081 ThrGlyAspAsnThrThrSerSerGluProAlaProSerGlyCysProProAspSer
 ACGGGCGACAATACGACAACATCCTCTGAGCCCCCCCCTCTGGCTGCCCTCCGACTCC
 TGCCCCCTGTTATGCTGTTAGGAGACTCGGGGGGGAAAGACCGACGGGGGGCTGAGG

 7141 PheAla
 AspAlaGluSerTyrSerSerMetProProLeuGluGlyGluProGlyAspProAspLeu
 GACGCTGAGTCCTATTCCCTCATGCCCTGGAGGGGAGCCTGGGATCCGGATCTT
 CTGCGACTCAGGATAAGGAGGTACGGGGGGACCTCCCCCTCGGACCCCTAGGCCTAGAA

 7201 SerAspGlySerTrpSerThrValSerSerGluAlaAsnAlaGluAspValValCysCys
 AGCGACGGGTCAAGGTCAAGGTCACTGAGTGGAGGCCAACGCGGGAGGATGTCGTGCTGC
 TCGCTGCCCAAGTACCACTGCCAGTCATCACTCCGGTTGCCCTCACAGCACACGACG

 7261 SerMetSerTyrSerTrpThrGlyAlaLeuValThrProCysAlaAlaGluGlnLys
 TCAATGTCTTACTCTTGGACAGGCGCACTCGTCACCCCGTGCAGCGGGAAAGAACAGAAA
 AGTTACAGAAATGAGAACCTGTCCCGTGTGAGCAGTGGGGCACGCGCGCCCTTGTCTTT

 7321 LeuProIleAsnAlaLeuSerAsnSerLeuLeuArgHisHisAsnLeuValTyrSerThr
 CTGCCCATCAATGCACTAACGCAACTCGTTGCTACGTCAACCACAATTGGGTATTCCACC
 GACGGGTAGTTACGTGATTGAGCAACGATGCACTGGTGTAAACCACATAAGGTGG

 7381 ThrSerArgSerAlaCysGlnArgGlnLysLysValThrPheAspArgLeuGlnValLeu
 ACCTCACGCGAGTGTGCTTGCACAAAGGAGAACAGTACATTGACAGACTGCAAGTCTG
 TGGAGTGCAGTCACGAAACGGTTCCGTCTTCACTGTGAAACTGTCTGACGTTCAAGAC

7441 AspSerHisTyrGlnAspValLeuLysGluValLysAlaAlaAlaSerLysValLysAla
 GACAGCCATTACCAGGACGTACTCAAGGAGGTTAAGCAGCGCGTCAAAGTGAAGGCT
 CTGTCGGTAATGGTCTGCATGAGTTCTCCAATTCTCGCCGAGTTTCACTTCCGA

Phe

7501 AsnLeuLeuSerValGluGluAlaCysSerLeuThrProProHisSerAlaLysSerLys
 AACTTGCTATCCGTAGAGGAAGCTTGCAGCCTGACGCCACACTCAGCCAAATCCAAG
 TTGAACGATAGGCATCTCTCGAACGTCGGACTGCAGGGGTGTGAGTCGGTTAGGTTC

PheGlyTyrGlyAlaLysAspValArgCysHisAlaArgLysAlaValThrHisIleAsn

7561 TTTGGTTATGGGGCAAAAGACGTCCGTGCCATGCCAGAAAGGCCGTAACCCACATCAAC
 AAACCAATACCCCGTTCTGCAGGCAACGGTACGGTCTTCCGGCATGGGTAGTTG

SerValTrpLysAspLeuLeuGluAspAsnValThrProIleAspThrThrIleMetAla

7621 TCCGTGGAAAGACCTCTGGAAAGACAATGTAACACCAATAGACACTACCATCATGGCT
 AGGCACACCTTCTGGAAAGACCTCTGTTACATTGTGGTTATCTGTGATGGTAGTACCGA

LysAsnGluValPheCysValGlnProGluLysGlyGlyArgLysProAlaArgLeuIle

7681 AAGAACGAGGTTCTCGCCTCAGCTGAGAAGGGGGTCGTAAGCCAGCTCGTCTCATC
 TTCTTGCTCCAAAAGACGCAAGTCGGACTCTCCCCCAGCACTGGTCGAGCAGTAG

ValPheProAspLeuGlyValArgValCysGluLysMetAlaLeuTyrAspValValThr

7741 GTGTTCCCCGATCTGGCGTGCCTGCGAAAGATGGTTTGACGACGTGGTTACA
 CACAAGGGCTAGACCCGCACCGCACACGCTTTCTACCGAACATGCTGCACCAATGT

LysLeuProLeuAlaValMetGlySerSerTyrGlyPheGlnTyrSerProGlyGlnArg

7801 AAGCTCCCTTGGCCGTGATGGGAAGCTCTACGGATTCCAATACTCACCAGGACAGCGG
 TTGAGGGGAACCGCACTACCCCTCGAGGATGCCAAGGTTATGAGTGGTCTGTCGCC

ValGluPheLeuValGinAlaTrpLysSerLysThrProMetGlyPheSerTyrAsp

7861 GTTGAATTCTCTCGTCAAGCGTGGAACTCAAGAAAACCCCAATGGGTTCTCGTATGAT
 CAACCTAAGGAGCACGTTGCACCTTCAGGTTCTTGGGTTACCCCAAGAGCATACTA

ThrArgCysPheAspSerThrValThrGluSerAspIleArgThrGluGluAlaIleTyr

7921 ACCCGCTGCTTTGACTCCACAGTCACTGAGAGCGACATCGTACGGAGGAGGCAATCTAC
 TGGCGACGAAACTGAGGTGTCAGTACTCGCTGTAGGCATGCCCTCCGGTAGATG

GlnCysCysAspLeuAspProGlnAlaArgValAlaIleLysSerLeuThrGluArgLeu

7981 CAATGTTGTGACCTCGACCCCCAAGCCCGTGGCCATCAAGTCCTCACCGAGAGGCTT
 GTTACAACACTGGAGCTGGGGGTTCGGCGCACCGTAGTTCAAGGGAGTGGCTCTCCGAA

Gly

8041 TyrValGlyGlyProLeuThrAsnSerArgGlyGluAsnCysGlyTyrArgArgCysArg
 TATGTTGGGGCCCTCTTACCAATTCAAGGGGGAGAACTGCGGCTATCGCAGGTGCCGC
 ATACAACCCCGGGAGAATGGTTAAGTCCCCCTCTGACGCCATAGCGTCCACGGCG

AlaSerGlyValLeuThrThrSerCysGlyAsnThrLeuThrCysTyrIleLysAlaArg

8101 GCGAGCGCGTACTGACAACTAGCTGTTAACACCCTCACCTGCTACATCAAGGCCCG
 CGCTCGCCGCATGACTGTTGACACACCATTGTGGGAGTGAACGATGTAGTTCCGGCC

AlaAlaCysArgAlaAlaGlyLeuGlnAspCysThrMetLeuValCysGlyAspAspLeu

8161 GCAGCCTGTCGAGCCGCAAGGGCTCCAGGAATGCACCATGCTGTGTGGCGACGACTTA
 CGTCGGACAGCTGGCGTCCCGAGGTCTGACGTGGTACGAGCACACACCGCTGCTGAAT

ValValIleCysGluSerAlaGlyValGlnGluAspAlaAlaSerLeuArgAlaPheThr

8221 GTCGTTATCTGTGAAAGCGCGGGGGTCCAGGAGGACGCGCGAGCCTGAGAGCCTTCACG
 CAGCAATAGACACTTTCGCGCCCCCAGGTCTCGCCGCTCGGACTCTCGGAAGTGC

GluAlaMetThrArgTyrSerAlaProProGlyAspProProGlnProGluTyrAspLeu

8281 GAGGCTATGACCAGTACTCCGCCCCCTGGGACCCCCCAACACAGAACATCGACTTG
 CTCCGATACTGGTCATGAGGGGGGGACCCCTGGGGGTGTGGTCTATGCTGAAC

GluLeuIleThrSerCysSerAsnValSerValAlaHisAspGlyAlaGlyLysArg

8341 GAGCTATAACATCATGCTCCTCAACGTGTCAGTCGCCACGACGGCGCTGGAAAGAGG
 CTCGAGTATTGTAGTACGAGGAGGTGACAGTCAGCGGGTGCTGCCCGACCTTCTCC

Fig. 17-9

8401 ValTyrTyrLeuThrArgAspProThrThrProLeuAlaArgAlaAlaTrpGluThrAla
 GTCTACTACCTCACCCGTGACCCTACAACCCCCCTCGCGAGAGCTGCGTGGGAGACAGCA
 CAGATGATGGAGTGGCACTGGATGTTGGGGAGCGCTCGACGCACCTCTGCGT

 8461 ArgHisThrProValAsnSerTrpLeuGlyAsnIleIleMetPheAlaProThrLeuTrp
 AGACACACTCCAGTCATTCTGGCTAGGCAACATAATCATGTTGCCAACACTGTGG
 TCTGTGTGAGGTCAAGTAAAGGACCGATCCGTTGTATTAGTACAAACGGGGTGTGACACC

 8521 AlaArgMetIleLeuMetThrHisPhePheSerValLeuIleAlaArgAspGlnLeuGlu
 GCGAGGATGATACTGATGACCCATTCTTCTGCGCCTTATAGCCAGGGACCAGCTTGAA
 CGCTCCTACTATGACTACTGGTAAAGAAATCGCAGGAATATCGGTCCCTGGTCGAACCT

 8581 GlnAlaLeuAspCysGluIleTyrGlyAlaCysTyrSerIleGluProLeuAspLeuPro
 CAGGCCCTCGATTGCGAGATCTACGGGGCCTGCTACTCCATAGAACCACTTGATCTACCT
 GTCCGGGAGCTAACGCTCTAGATGCCCGGACGATGAGGTATCTGGTGAACTAGATGGA

 8641 ProIleIleGlnArgLeuHisGlyLeuSerAlaPheSerLeuHisSerTyrSerProGly
 CCAATCATTCAAAGACTCCATGGCCTCAGCGCATTTCACTCCACAGTTACTCTCCAGGT
 GGTTAGTAAGTTCTGAGGTACCGGAGTCGCGTAAAGTGAAGGTGTCAATGAGAGGTCCA

 8701 GluIleAsnArgValAlaAlaCysLeuArgLysLeuGlyValProProLeuArgAlaTrp
 GAAATTAAATAGGGTGGCCGCATGCCTCAGAAAACCTGGGGTACCGCCCTTGCAGCTTGG
 CTTAATTATCCCACCGCGTACGGAGTCTTGAACCCATGGCGGGAACGCTCGAACCG

 Gly
 8761 ArgHisArgAlaArgSerValArgAlaArgLeuLeuAlaArgGlyGlyArgAlaAlaile
 AGACACCAGGGCCGGAGCGTCCCGCCTAGGCTCTGGCCAGAGGAGGCAGGGCTGCCATA
 TCTGTGCCCGGGCCTCGCAGGCGCGATCCGAAGACCGGTCTCCCTCCGACGGTAT

 8821 CysGlyLysTyrLeuPheAsnTrpAlaValArgThrLysLeuLys
 TGTGGCAAGTACCTCTTCAACTGGGCAGTAAGAACAAAGCTCAAAC
 ACACCGTTCAAGGAGAAGTTGACCCGTCAATTCTTGTGAGTTG

Fig. 17-10

IMMUNOLOGICAL SCREENING IN BACTERIA

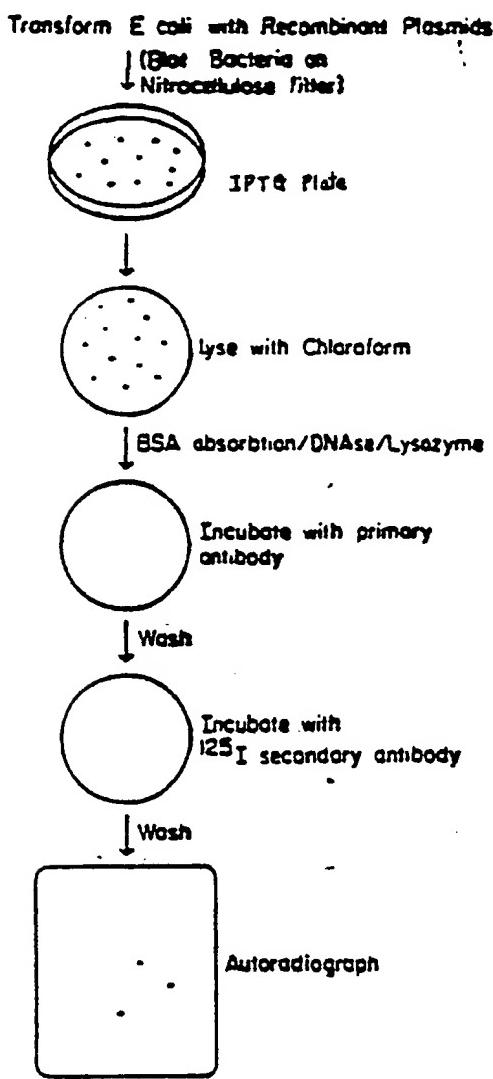


FIGURE 18

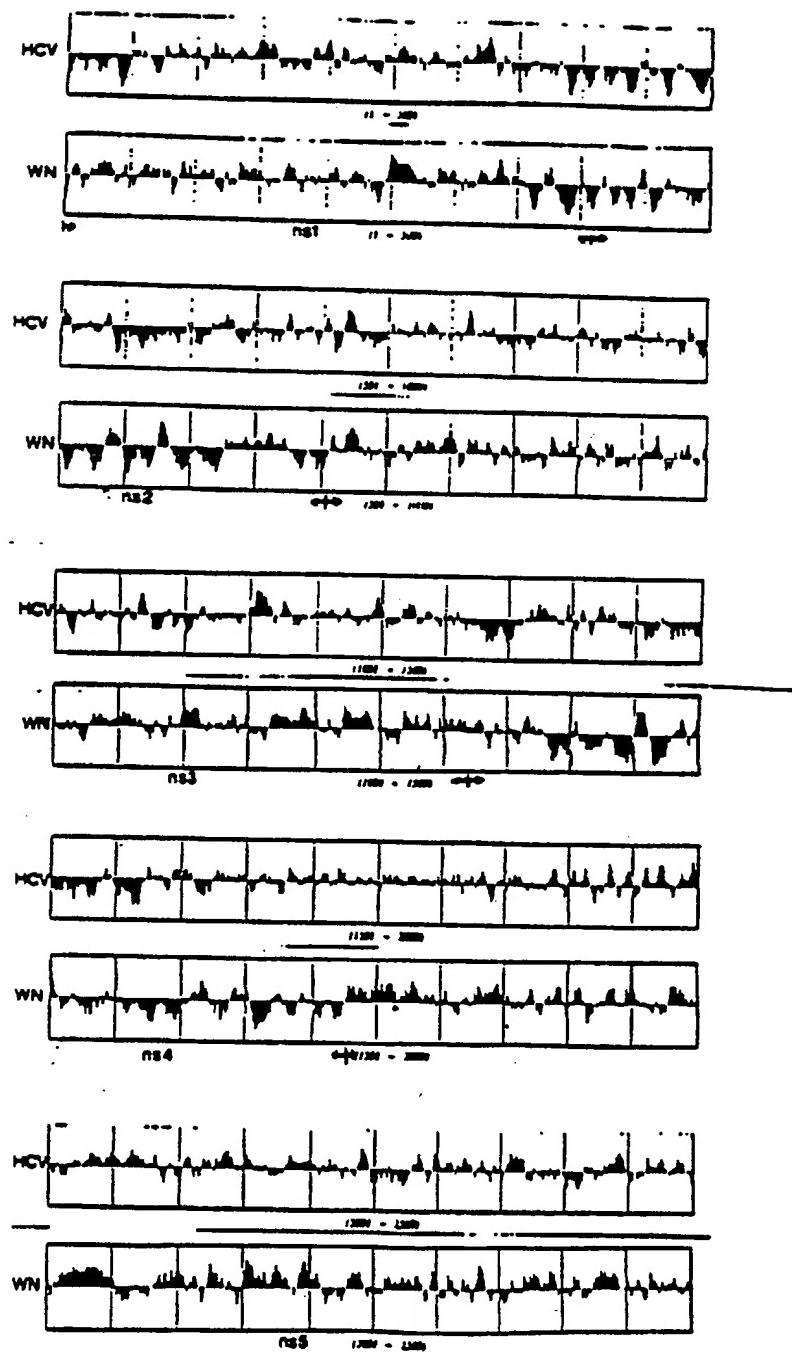


FIGURE 19

EP 0 388 232 A1

HCV ($n = 500$)

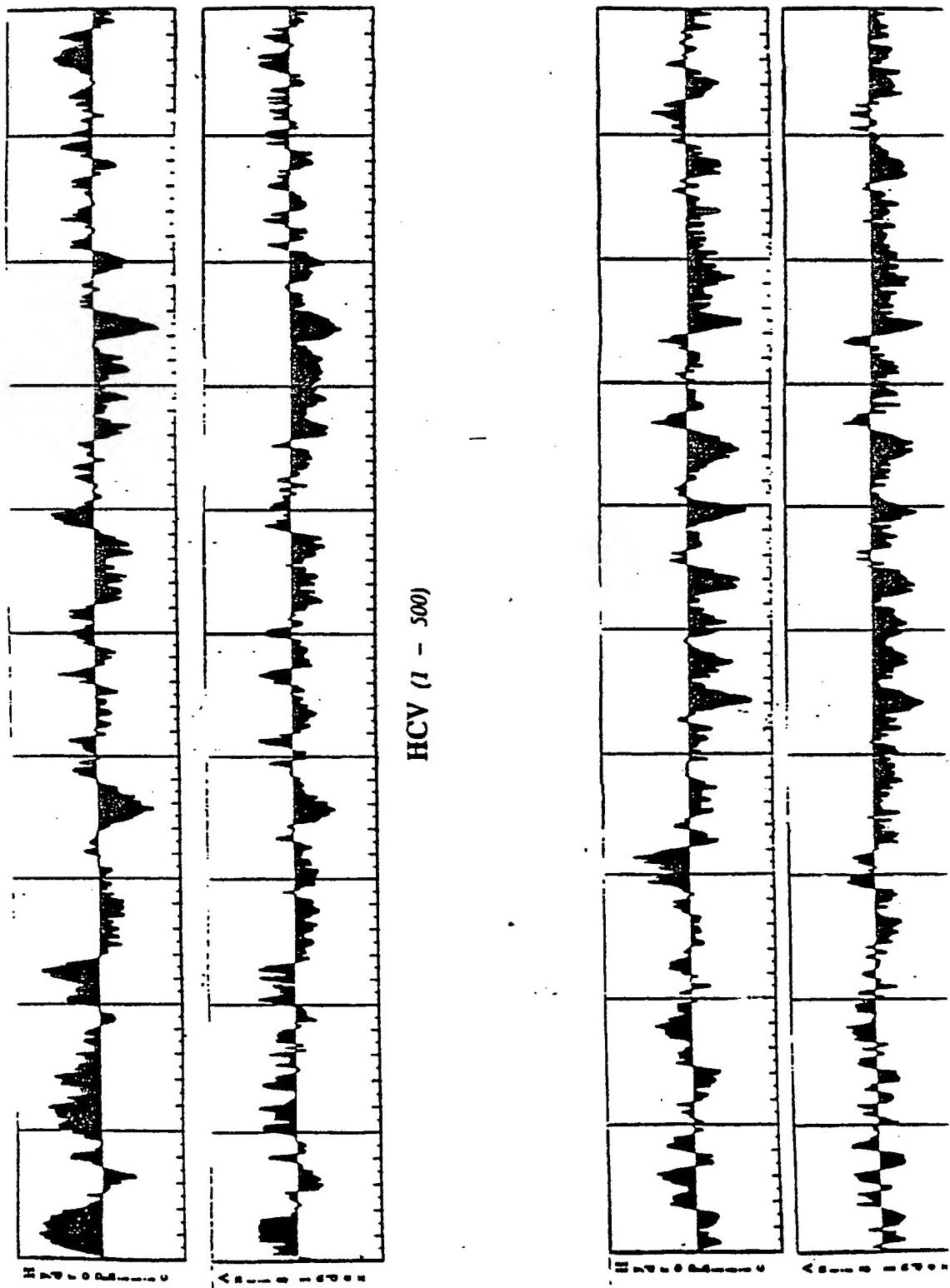
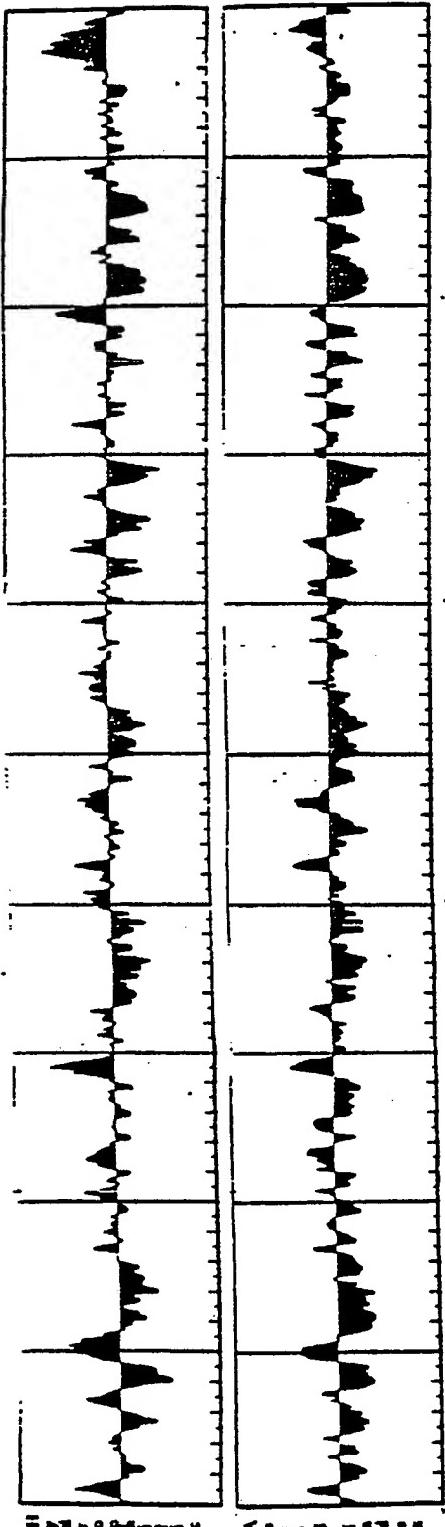


FIGURE 20-1

EP 0 388 232 A1



HCV (1001 - 1500)

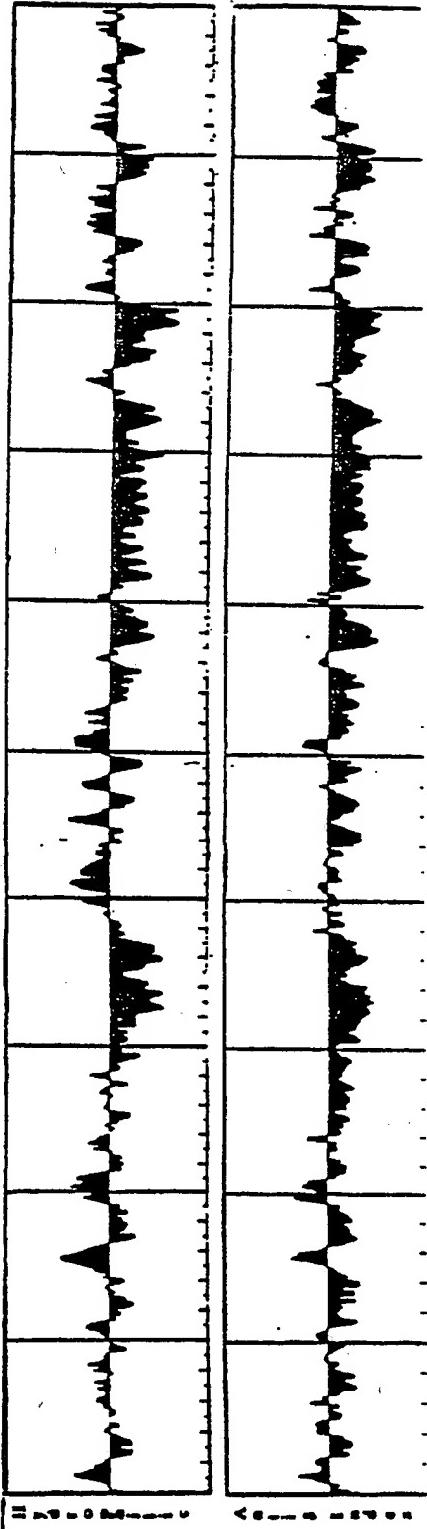
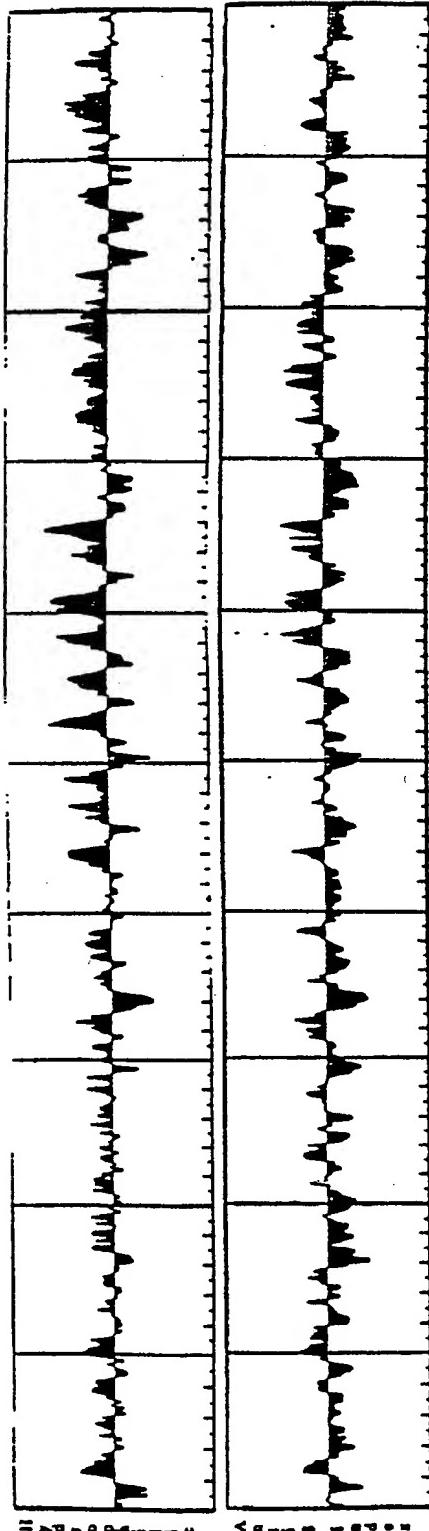


FIGURE 20-2

EP 0 388 232 A1



HCV (2001 - 2500)

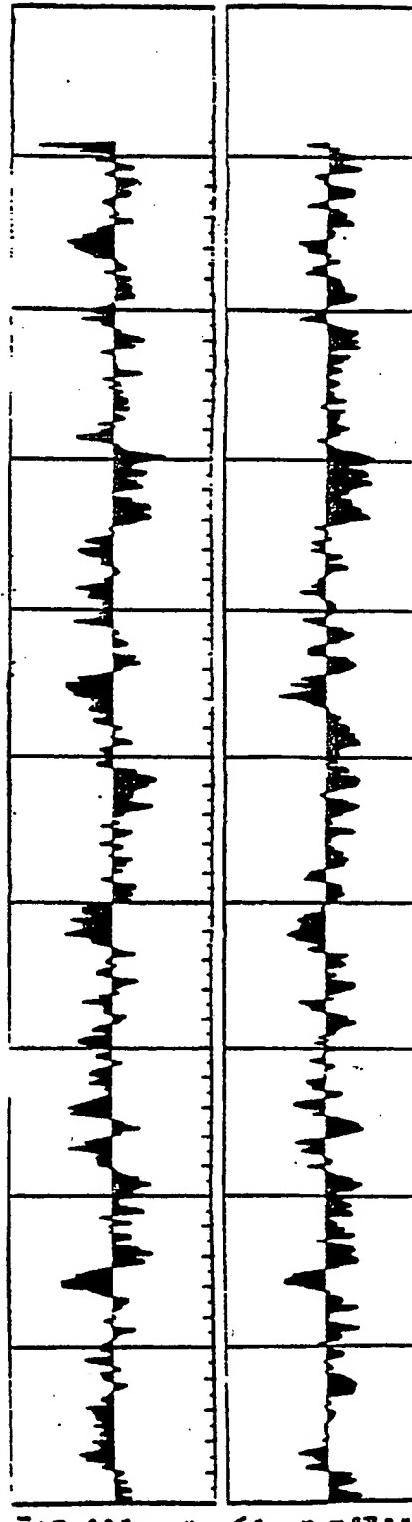


FIGURE 20-3

Some conserved co-linear peptides in HCV & Flaviviruses

Flaviviruses (Yellow Fever, West Nile, Dengue)	NS3 region	NS5 Highly-conserved Polymerase region
	TATPPG-----SMAQRRGRIGRNP-----GDDCVV	
	***** * ***** *	*** **
HCV	TATPPG-----SRTQRGRGTGRGK-----GDDCVV	
	1348 1483	12737

FIGURE 21